

ANNALS OF THE NEW YORK ACADEMY OF SCIENCES

VOLUME 74, ART. 3 PAGES 407-1028

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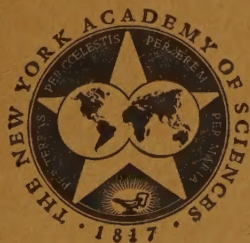
CHLORPROPAMIDE AND DIABETES MELLITUS

BY

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MARTIN G. GOLDNER



NEW YORK

PUBLISHED BY THE ACADEMY

March 30, 1959

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* This series of papers is the result of a conference on *Chlorpropamide and Diabetes Mellitus*, held and supported conjointly by The New York Academy of Sciences and Chas. Pfizer & Co., Inc., Brooklyn, N.Y., on September 25, 26, and 27, 1958.

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PREFACE

Albert S. Gordon

*Department of Biology, Graduate School of Arts and Science,
New York University, New York, N. Y.*

A new era in the field of diabetes mellitus has been ushered in with the introduction of effective oral hypoglycemic agents, particularly the sulfonylureas. The great impact made by these agents was evident in the success attending the conference on *Sulfonylureas and Related Compounds* sponsored by The New York Academy of Sciences in February 1957. It was apparent from this conference that compounds such as tolbutamide are to be added to the armamentarium useful in the management of diabetes. These substances appear capable of controlling the condition in adult-onset diabetes with considerable success, provided precautions of dietary control similar to those utilized for insulin therapy are also followed.

The first conference emphasized the significance of the sulfonylureas, not only because of their use in the treatment of diabetic states, but also because they served to rekindle interest and further research in the etiology of diabetes, in the mechanisms of insulin synthesis, storage and release, and in the role of the liver and peripheral tissues in carbohydrate metabolism.

The present conference centers about sulfonylurea derivatives, particularly chlorpropamide. It is becoming increasingly evident that incorporation of members of the halogen series into the structure of organic molecules often enhances their biological activities. This is true of hormones of adrenocortical origin in which, for example, substitution of halogens such as fluorine into the 9- and 6- α positions results in considerable augmentation of certain physiological actions. A similar situation is seen in the synthesis of thyronine derivatives in which iodine is superior to bromine and chloride. This concept also extends to the sulfonylurea compounds, notably chlorpropamide.

We look forward to further detailed information on topics relating to the mechanism of action of the sulfonylureas, particularly their influence on insulin metabolism; to a comparison of the relative effectiveness and toxicity of the newer derivatives, especially chlorpropamide; and to the extension of their use in the treatment of diabetic states in man. All of these topics are discussed in this monograph.

INTRODUCTORY REMARKS

Martin G. Goldner

Jewish Chronic Disease Hospital, Brooklyn, N. Y.

It is my hope that the presentations and discussions comprising this monograph will bring us another step forward in the understanding of the role that the hypoglycemic sulfonylurea derivatives can play in the management of diabetes mellitus.

When in 1942 the first chance observations were made at the University of Montpellier in southern France about the hypoglycemic action of a new sulfonamide, Loubatières foresaw that at last the long and so far rather fruitless search for an oral drug for the treatment of diabetes mellitus had reached a new phase and that a promising agent was finally at hand.

However, it took another chance observation, this time by German investigators, and another decade until the first such drugs—tolbutamide and carbutamide—could be introduced into the practice of medicine. During the past few years nearly 1000 other sulfonylurea derivatives have been developed and evaluated in various laboratories. Chlorpropamide, or Diabinese, is one of these compounds. Because of such special characteristics of this preparation as its effectiveness in rather small doses and its lasting action, it was given an extended clinical tryout. The reports of these trials included in these pages will give us an opportunity to decide whether a new and practically useful agent has come into our hands in addition to those presently available.

Part I. Pharmacological Studies

GENERAL PHARMACODYNAMICS OF THE HYPOGLYCEMIC ARYLSULFONAMIDES

Auguste Loubatières

*Laboratory of Applied Physiology, Faculty of Medicine,
Institute of Biology, Montpellier, France*

The question of the general pharmacodynamics of the hypoglycemic arylsulfonamides, of which the following* is a brief survey, has been the subject of a number of our more detailed articles.¹⁻¹⁰ We shall discuss here only the essential points in regard to this important question.

First, we should record the fact that the basic experimental discoveries made in our laboratory between 1942 and the present time have been confirmed by numerous authors using either our compounds 2-(*p*-aminobenzene-sulfamido)-5-isopropyl-1-thio-3,4-diazole (RP 2254) and 2-(*p*-aminobenzene-sulfamido)-5-*tert*.-butyl-1-thio-3,4-diazole (RP 2259, glybuthiazole) or using their congeners 1-butyl-3-sulfanilylurea (BZ 55, carbutamide) and 1-butyl-3-*p*-tolylsulfonylurea (D 860, tolbutamide).

The compound 1-propyl-3-(*p*-chlorobenzenesulfonyl)urea (P 607), chlorpropamide, is still under study, and at present some reservations must be expressed regarding it. Although its mechanism of action is probably the same as that of the compounds mentioned above, this is not established; also, we are not well informed as to its toxicity and tolerance. It is the purpose of this monograph to contribute toward establishing all these points.

All of these compounds are arylsulfonamides; that is to say, aromatic sulfonamides. In designating them here we shall use the terms "sulfamide," "sulfonamide," or "aryl-sulfonamide" as seems suitable.

Mechanism of Action: Experimental Study

The fundamental and elemental role played by the endocrine pancreas (the beta cells of the islets of Langerhans) and insulin characterizes the mechanism of action of the arylsulfonamides.

As long ago as 1944 we demonstrated that the sulfonamides do not produce hypoglycemia in the totally depancreatized dog but that, if even a fraction of the pancreas containing some islets of Langerhans is left in place, the hypoglycemic action of these substances is manifested. When these substances are introduced into the organism they come into contact, by way of the blood stream, with the islets of Langerhans for which they have a special tropism; they are pancreatropic.

They act as stimulants to the beta cells from which they release a certain amount of endogenous insulin into the blood. Numerous experimental deductions support this statement.

* This account is a revised version of that presented at the meeting of the Third International Congress of the International Diabetes Federation held from July 21 to 25, 1958, at Düsseldorf, Germany.

The effects of these compounds are as follows: (1) the beta cells lose some of their intraprotoplasmic insulin granules; (2) the insulin content of the pancreatic tissue diminishes in response to the development of glycemia; (3) as shown by us in 1946 by means of experimental pancreatic-jugular anastomosis, the hypoglycemic activity of the blood leaving the pancreas increases considerably: a fact since confirmed in several ways, especially by measuring the insulin activity of the blood plasma leaving the pancreas.

Recently we resumed these experiments and showed that the venous blood leaving the pancreas of a normal dog, transfused into a second normal dog acting as reagent, is more pronouncedly hypoglycemic and, especially, is hypoglycemic longer during the action of the sulfonamide RP 2259 than before the action of the drug.

In severe alloxan diabetes the sulfonamides are inactive, since almost all of the beta cells are destroyed. On the other hand, in alloxan diabetes of moderate intensity, in which a certain number of beta cells have been spared, the sulfamides are hypoglycemic.

It is also known that several days of sulfonamide administration produce in the normal animal a hyperplasia of the islet beta cell system and stimulate the formation of new beta cells that come into being at the expense of cells of the exocrine pancreatic gland (the centroacinar cells, cells of the excretory canaliculi, the acinar cells). Here is a demonstration of the validity of Laguesse's theory that the cells of the exocrine gland may be the origin of endocrine cellular elements.

In 1956 we studied the roles of the endocrine glands other than the pancreas in the mechanism of action of the arylsulfonamides. The following experiments carried out in our laboratory demonstrate the fundamental role of the pancreas.

Using the same dog, we removed, in several operative procedures, the following endocrine glands, one after another, in this order: (1) the two adrenals (cortical and medullary tissues); (2) the thyroid, the four parathyroids, and the genital glands; and (3) the entire hypophysis.

After each of the operative procedures the sulfonamide proved to be hypoglycemic, except following the removal of the entire pancreas. However, it is clear that hypophysectomy and, especially, adrenalectomy sensitize the organism to the hypoglycemia induced by the sulfamides. The sulfonamides retain their hypoglycemic action in the hepatectomized animal, provided the pancreas is intact. However, this does not imply that the liver plays no role.

The glycogenic action that these compounds exert upon the liver, demonstrated by us in 1944, is the basis for postulating that the sulfonamides act by inhibiting certain enzymes (glucose-6-phosphatase; reactivation of phosphorylase). However, these effects do not seem to be specific and are manifest only at high concentrations of the drugs. Moreover, the inhibition they produce is only partial.

Our recent experiments upon the dog subjected to a preliminary eight-day fast, which slows insulin secretion, show clearly that liver glycogen formation can be explained by the effects on the liver cell of endogenous insulin released by the sulfonamide. Furthermore, we observed that administration of

sulfonamide also induces an accumulation of glycogen in muscle, that is to say, it affects the peripheral tissues. Both of these results, the hepatic and the muscular, of the sulfonamides may be reproduced completely by physiological amounts of insulin.

In addition, we have been able to show that sulfonamide does not oppose the considerable diminution of liver glycogen that follows the suppression of the pancreas or of insulin secretion in the dog. Lastly, when sulfonamide is present, the glycogenic action of insulin exerted on the liver of the totally depancreatized dog is preserved completely. These facts show that the sulfonamides do not seem to inhibit profoundly the enzymatic reactions involved in glycogenolysis or hepatic glycogen formation in the physiological conditions under which we operated.

Furthermore, we showed at the same time as Houssay and his collaborators that the sulfonamides in massive doses potentiate the hypoglycemic effects of insulin, even after ablation of the pancreas. It has not been demonstrated that this potentiation of the effects of insulin is due to an inhibition of insulinase. We have other hypotheses. Whatever the explanation, the release of endogenous insulin precedes the subsequent intensification of the effects of this hormone. A possible action of the sulfonamides on the peripheral tissues, in the absence of insulin or the pancreas, has not been demonstrated. Recent study of the peripheral arteriovenous differences in blood sugar after sulfonamide and after insulin shows striking similarities.

On the other hand, we have shown that the administration of sulfonamide is capable of accumulating glycogen in the striated muscle of a dog subjected to prolonged preliminary fasting. We have reason to believe that endogenous insulin released by the sulfonamide and not wholly arrested by the hepatic barrier is responsible for these peripheral effects.

In addition to their hypoglycemic action, the sulfonamides possess an antidiabetic action that is exhibited under certain experimental conditions in the dog with alloxan diabetes. We have shown that, if the diabetes is severe (that is to say, if all of the beta cells have been destroyed by the alloxan), the sulfonamides are inactive; they may even hasten the evolution of the diabetes toward acidosis.

On the other hand, if the diabetes is of moderate intensity, the action of the sulfonamides is evident. The different manifestations of the diabetes progressively diminish and then disappear. After several weeks the treatment can be discontinued, and the animal is "cured." New beta cells have developed in the pancreas of these animals. We have shown that these cells are functionally normal, since they are perfectly capable of regulating the blood sugar; they are therefore sensitive to the delicate stimuli for insulin secretion. They can be stimulated by the sulfonamides and, in turn, they can be destroyed by alloxan.

This new islet apparatus, whose functional activity exists thanks to the action of the sulfonamides, regulates the blood sugar perfectly if the animal is fasting. A balanced diet allows the "cure" to be maintained for a year or more. However, excessive and extended feeding renders the animal progressively hyperglycemic by the end of six months. This shows the impor-

tance of a controlled diet for maintaining the benefits obtained from the sulfonamides.

Mechanism of Action in Man

Can proof of hypoglycemic antidiabetic action be established in normal or diabetic man? The hypoglycemic action of the sulfonamides is admitted without reservation in the case of normal man. On rare occasions these substances cause serious hypoglycemic accidents that, in any case, are reversible by glucose. In a man totally without pancreatic tissue these substances are inactive; they maintain their activity if a small amount of endocrine pancreas remains in the organism.

Hypophysectomy and adrenalectomy do not affect hypoglycemic action. Hyperfunction of the endocrine glands producing hypoglycemic or diabetogenic secretions opposes or modifies the action of the sulfonamides on blood sugar. The effects observed depend, therefore, on the respective and opposite roles played in establishing the total diabetic state by the pancreatic component, on the one hand, and the extrapancreatic diabetogenic component on the other. This explains the variable results observed following the use of sulfonamides when glandular hyperfunction (anterior pituitary or adrenal cortical) is beginning, or when this hyperfunction is being reduced. The hemosiderin depot observed in the beta cells of the islets of Langerhans probably is not unrelated to the lack of action of the sulfonamides in hemochromatosis (bronze diabetes). This behavior confirms once more the tropism of the sulfonamides for the beta cells of the pancreas.

In cirrhosis of the liver with diabetes, the activity of the sulfonamides is variable and depends on many factors. In the diabetic man under insulin treatment, the simultaneous administration of sulfonamide permits, in certain cases, the reduction of the dosage of injected pancreatic hormone. Is this a phenomenon of potentiation? Has the endogenous insulin released by the sulfonamide added its effects to that of the injected insulin? Do other mechanisms enter in? These questions are still unanswered.

We have recently answered several questions in regard to sulfamide treatment. It has been stated, for example, that the sulfonamides are not physiological agents. That is true, but their pharmacological action causes endogenous insulin secretion; that is to say, of a hormone that, in its physiological route of action, is poured into the blood of the portal vein to act first on the liver and then on the peripheral cells by way of the general circulation. There is reason to believe that, when sulfamide treatment is maintained, stimulation of endocrine insulin secretion is continuous. It is evident that this continuity of secretion is particularly beneficial for glycemic regulation and metabolism.

The efficacy of the sulfonamides in ketosis (the presence in small quantity of ketone bodies in the organism) and their inefficacy in severe acidosis is explained by the degree of lesion of the beta islet cells of the pancreas in the different stages that represent the various degrees of intensity of the diabetes.

The classification of diabetic subjects in terms of their different responses to the sulfamides is a strong argument in favor of our theory of the mechanism of action of these substances. Young diabetics, refractory to the sulfamides,

have severe beta cell lesions in their pancreas; the insulin content of this organ is low; the stimulation of its cells by glucose administration releases practically no detectable insulin into the blood.

On the other hand, diabetics stricken with the disease at a mature age have numerous intact beta cells in their pancreas; the insulin content of this organ represents 50 to 60 per cent of normal; glucose administration shows that the cells are capable of stimulation and of liberating (although in a little less than normal quantity) insulin into the blood. It can therefore be acknowledged that the sulfonamides seem to act especially in subjects exhibiting a certain degree of sluggishness or insufficiency of the insulin-secretory processes. Despite the considerable amount of work accomplished since 1946, the theory that emerges remains in conformity with the one we have maintained since then and will continue to maintain, because it is well founded.

There is a question as to whether the chronic stimulation of the beta cells may not finally bring on their exhaustion and an aggravation of the diabetes. Taking into consideration our long experience, it does not seem that this contingency often arises when the sulfonamides are correctly used in subjects who are really suitable. When this condition arises, it is necessary to take into account deviations from the diet, the way in which the sulfonamides are used, and the spontaneous aggravative evolution of certain cases of diabetes, before the sulfamides themselves can be held entirely responsible. Conditions called "refractory" to the sulfonamides may appear; they may be explained in part by the phenomenon of tachyphylaxis.

Hypoglycemic Sulfamides and the Prospects for Extended Remission of Diabetes Mellitus

In conclusion we should like to explain why, in our opinion, the hypoglycemic and antidiabetic sulfamides offer favorable prospects for prophylaxis or extended remission of diabetes.

Accumulated clinical data show two types of results: in certain elderly diabetics or even in young diabetics (provided their diabetes is recent and not severe) a sulfamide treatment carried on for several weeks can cause the diabetes to disappear. In many cases the benefits last for several months after suspension of sulfamide treatment. The sulfamides have, therefore, an antidiabetic action in man as well as in animals.

It is possible that the extended remission of diabetes, which is not a final cure, is explained by at least partial regeneration of the endocrine pancreatic system. If this is true, why not attempt to use the sulfonamides as prophylactic and retarding agents in subjects predisposed to this disease?

There is another point that should engage our attention, since it presents a problem and indicates a line of research. It involves the observation of differences in glycemic behavior: on the one hand, in the animal with alloxan diabetes "cured" by the sulfonamides and, on the other hand, in the diabetic man with extended remission of diabetes due to sulfonamide treatment. In the "cured" animal the blood sugar is normal and, if the diet is correctly controlled, the "cure" lasts. Lesions caused by alloxan were pancreatic;

the sulfonamides have compensated for the pancreatic lesions, at least in part, by facilitating the regeneration of the beta cells; glycemic equilibrium has returned and will be prolonged if certain experimental conditions are respected. In extended remission of diabetes in man, blood sugar is almost never normal, but is definitely above 1.10 to 1.20 gm. of glucose per liter. After several weeks or months the diabetes progressively resumes with all its former intensity. What has happened?

The sulfamide treatment, it is true, has partially restored the endocrine pancreatic gland, but it has not suppressed the extrapancreatic diabetogenic (hypophyseal, adrenal) influences that will bear down with all their weight on a particularly fragile islet beta cell system and will cause the reinstatement of diabetes.

Thanks to the research accomplished in the domain of the hypoglycemic sulfonamides, an important advance has been made, but our task as seekers is not yet over. Now we must undertake to detect and neutralize the harmful extrapancreatic influences. It is probable that we shall not be too long without weapons against these factors. We should succeed in this because these influences often are exerted only temporarily in the course of a lifetime (period of somatic growth; premenopausal period), and science should not lose the hope of overcoming them once they are detected.

The "cure" of experimental diabetes is possible if the disease has not exceeded a certain degree of severity. It is not unreasonable to believe that we are on the way to realizing, within organically permitted limits at least, the extended remission of human diabetes mellitus. If we arrive at this result, the challenge offered by the hypoglycemic and antidiabetic sulfonamides sixteen years ago to diabetologists will not, from the scientific, the social, and the human points of view, have been in vain.

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* A detailed bibliography on the subject of hypoglycemic arylsulfonamides will be found in the author's published works.

STUDIES ON THE EFFECT OF ORAL ANTIDIABETIC COMPOUNDS ON GLUCOSE TOLERANCE, ON ISLET CELL STRUCTURE, AND ON THE *IN VITRO* METABOLISM OF ISOLATED MUSCLE

K. Lundbaek, K. Nielsen, O. J. Rafaelsen

*Second Department of Internal Medicine and Department of Pathology,
Aarhus University School of Medicine, Aarhus, Denmark*

The mode of action and the locus or loci of action of the individual oral antidiabetic compounds have not been fully elucidated. We shall present briefly the results of three kinds of studies in which we have tried to obtain some information about the action of these compounds.

Oral and Intravenous Glucose Tolerance During the Action of Oral Antidiabetic Compounds

The first study is of glucose tolerance in diabetic patients.¹ FIGURE 1 shows the average oral glucose tolerance curve before and during the administration of carbutamide or tolbutamide in 18 patients. The antidiabetic effect is

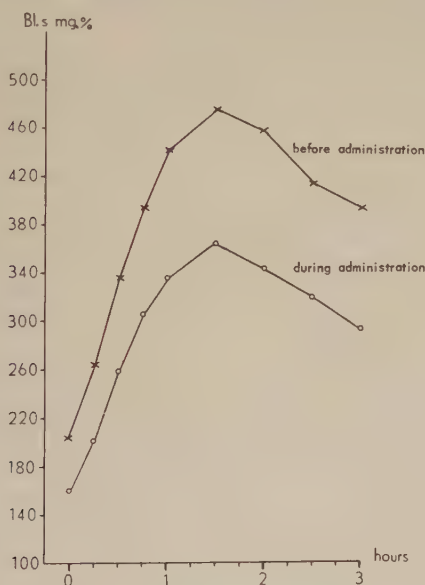


FIGURE 1. Average curves showing oral glucose tolerance before and during the administration of carbutamide or tolbutamide.

quite obvious. It is not only a parallel displacement of the curve that occurs; the area of increase, that is, the area above fasting level, is significantly decreased during the action of the sulfonylureas. FIGURE 2 shows the results of intravenous glucose tolerance tests. The individual k values,

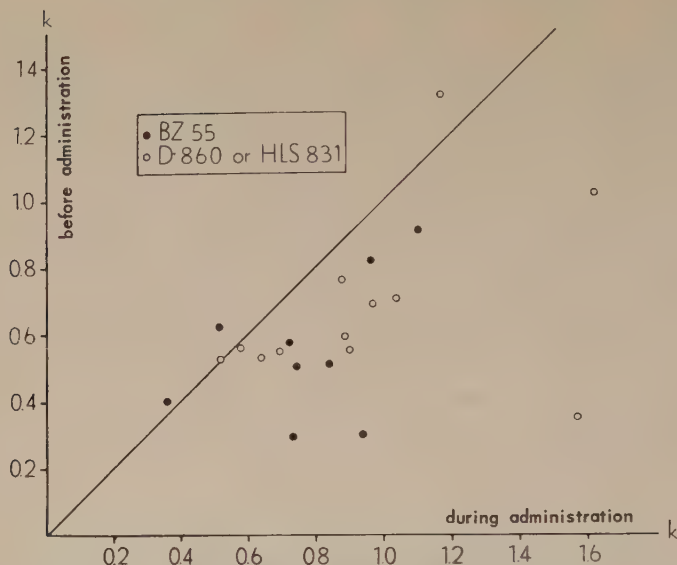


FIGURE 2. k Values for intravenous glucose tolerance curves before and during the administration of carbutamide (BZ 55) or tolbutamide (D 860 or HLS 831).

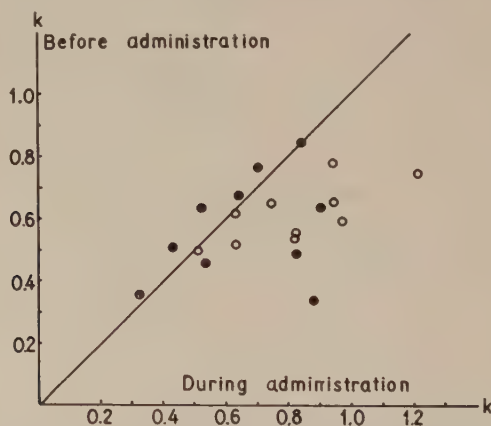


FIGURE 3. k Values for intravenous glucose tolerance curves before and during the administration of chlorpropamide. Symbols: \bullet = 1.75 gm. during a period of 48 hours. \circ = 3 to 5 gm. during a period of 48 to 96 hours.

expressing the slope of the decrease in blood sugar in each of the patients before and during treatment with sulfonylureas, are plotted against each other. An antidiabetic effect on the intravenous glucose tolerance is seen, the points clearly tending to accumulate below the 45° identity line.

These k values have been obtained by calculation on the basis of the original blood-sugar values. We have tried to calculate the k values from the so-called "blood-sugar excess," that is, blood sugar minus fasting blood

sugar.² Even by these devious ways we find that an antidiabetic effect, although less significant, can be shown to occur in the intravenous glucose tolerance test. Baird and Duncan³ have published findings very similar to ours but, by using the blood-sugar excess values, they failed to demonstrate any significant effect.

TABLE 1 shows the serum inorganic phosphate during glucose tolerance tests before and during the action of sulfonylureas. It appears that the

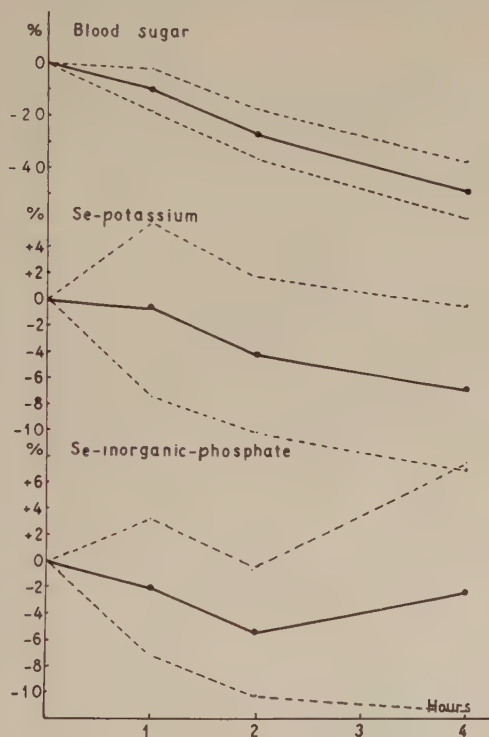


FIGURE 4. Blood sugar, serum potassium, and serum inorganic phosphate 1, 2, and 4 hours after oral administration of 1 gm. chlorpropamide. Values are shown as per cent changes from the "before" values. Dotted lines represent standard deviation.

amelioration of the glucose tolerance was not accompanied by any change in the phosphate response.

FIGURE 3 shows the results of intravenous glucose tolerance tests in 10 diabetic patients before and during administration of chlorpropamide. It appears that 1 gm./day of this compound produces an amelioration of the glucose tolerance similar to the one found with carbutamide or tolbutamide. However, chlorpropamide increased to some extent the response of the inorganic serum phosphate to glucose (TABLE 1) and, when given in the fasting state, it produced a small but significant fall of serum phosphate and of serum potassium (FIGURE 4).

TABLE 1
AVERAGE SERUM INORGANIC PHOSPHATE DURING ORAL AND INTRAVENOUS GLUCOSE TOLERANCE TEST BEFORE AND DURING THE ADMINISTRATION OF CARBUTAMIDE (C), TOLBUTAMIDE (T), OR CHLORPROPAMIDE (CP)

Glucose tolerance test	Antidiabetic compound	No. of patients	Time after oral or I.V. glucose (min.)				Max. fall	Percentage fall
			0	60	120	180		
Oral.....	0	18	3.8	3.6	3.5	3.4	0.41	10.8
Oral.....	C or T	18	3.7	3.4	3.3	3.4	0.41	11.1
Intravenous....	0	21	3.7	3.5				5.4
Intravenous....	C or T	21	3.6	3.4				5.6
Intravenous....	0	20	3.6	3.5				3.8
Intravenous....	CP	20	3.8	3.5				7.1

It is not clear if this difference between the response of the inorganic phosphate to carbutamide and tolbutamide, on the one hand, and to chlorpropamide, on the other, is due to a difference in the mode of action of these compounds. It should be noted that under certain circumstances a fall of serum inorganic phosphate has been reported to occur after administration of tolbutamide.^{4, 5}

Pancreatic Islets of the Rat During the Administration of Sulfonylureas

The second type of study concerns the histological structure of the pancreatic islets in the rat during the administration of sulfonylureas.⁶

Normal female rats were injected intraperitoneally with *p*-aminobenzene-sulfonamidoisopropylthiodiazole (IPTD), carbutamide, or tolbutamide in doses ranging between 50 and 450 mg. in a single injection; in other experiments 75 mg. were given daily over a period of from 3 to 45 days. The islet cells were stained with chrome-alum-hematoxylin-phloxin, closely following the details of the procedure described by Gomori.⁷ The results are shown in TABLE 2. No difference in the structure or granulation of the alpha or beta cells of the controls or of the injected animals was obtained. Synthalin A is seen to produce the classic alpha cell degeneration.

These results are not in agreement with the findings of Kracht and Rausch-Stroomann,⁸ who reported beta cell degranulation after tolbutamide injection in the rat. They differ in the same respect from the results of studies in the calf⁹ and in the rabbit.¹⁰ This has been interpreted as histological support for the hypothesis that the oral antidiabetic compounds act by increasing the production and/or output of insulin from the pancreatic islets. In our laboratory, working with rats, we have not been able to produce any histological evidence for this hypothesis.

However we emphasize that we have had considerable difficulty with the Gomori stainings, which seem to vary somewhat from day to day, giving

TABLE 2
HISTOLOGICAL CHANGES IN PANCREATIC ALPHA AND BETA CELLS OF RATS AFTER
ADMINISTRATION OF VARIOUS ANTIDIABETIC DRUGS

Drug	No. of animals	No. of controls	Total dose (mg.)	Histological changes of	
				Alpha cells	Beta cells
Carbutamide.....	75	29	75-3375	0	0
Tolbutamide.....	44	11	75-3375	0	0
IPTD.....	28	9	75-3375	0	0
Synthalin A.....	10	3	20	+++	0
Total.....	157	52	—	—	—

the illusion of a change in the granulation of the beta cells. We have found that it was always necessary to carry the control specimens in the same run all the way through the entire procedure.

In Vitro Studies of Isolated Rat Diaphragm

The third type of experiment deals with the action of sulfonylureas on the isolated diaphragm of the fasting rat.¹¹

For these experiments the usual Gemmil technique was used. The medium was Gey and Gey's buffer with 300 mg. per cent of glucose. The antidiabetic compounds were either injected into the animals a few hours before the experiment or were added to the medium. Each experiment included 6 to 12 treated and 6 to 12 control hemidiaphragms. In injection experiments we attempted to produce a blood level of about 10 to 50 mg. per cent of carbutamide. In additional experiments the medium contained from 6 to 100 mg. per cent of the antidiabetic compound.

In injection experiments with carbutamide and in experiments in which carbutamide, tolbutamide, IPTD, or phenethyldiguanide was added to the medium, a significant rise in the glucose uptake was observed consistently. No change occurred in the glycogen content of the muscle except with phenethyldiguanide, which produced a fall in the muscle glycogen.

The results of the experiments with antidiabetic compounds added to the medium are summarized in FIGURES 5 and 6.

Our findings demonstrate that IPTD, carbutamide, and tolbutamide can increase the uptake of glucose in the isolated muscle tissue without affecting glycogen deposition: that is, changes in the metabolism of the muscle that differ from the action of insulin on isolated muscle tissue. Similar results have been obtained by Canal *et al.*¹² and by Pletscher and Gey,¹³ but other investigators have reported negative results of such studies.¹⁴⁻¹⁷ Our results, using phenethyldiguanide, are in accordance with the results reported by Williams *et al.*¹⁸

The results obtained in our laboratory indicate that the action of the

antidiabetic compounds on the muscle is independent of the presence of active beta cells: that it is not brought about by an increase in the production and delivery of insulin from the pancreas.

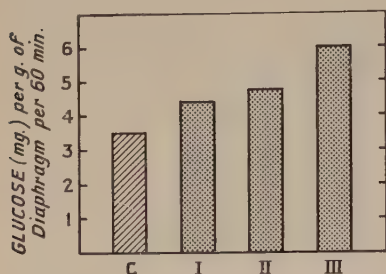


FIGURE 5. Average uptake of glucose by the isolated rat diaphragm incubated in glucose-containing medium with various oral antidiabetic compounds. C, controls; I, carbutamide; II, tolbutamide; III, phenethylidiguanide; concentration, 35 mg. per cent. The difference between each of these average values is statistically significant.

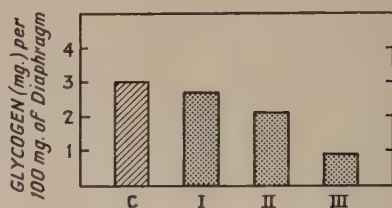


FIGURE 6. Average glycogen content of the isolated rat diaphragm after incubation in glucose-containing medium with various oral antidiabetic compounds. C, I, II, III, and concentration are as in FIGURE 5. III is significantly lower than C, I, and II.

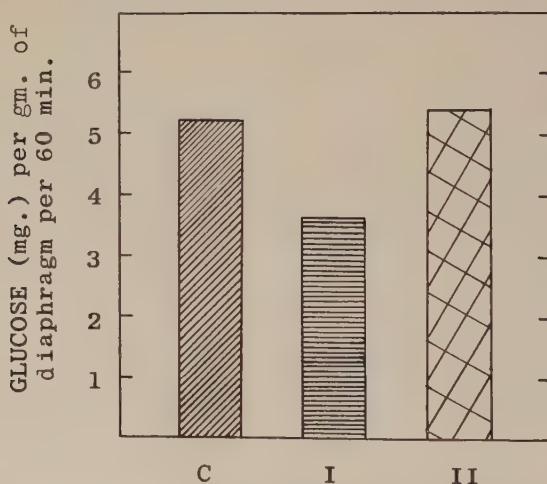


FIGURE 7. Average uptake of glucose by the isolated diaphragm of alloxan-diabetic rats. C, controls; I, alloxan-diabetic rats; II, alloxan-diabetic rats (concentration of carbutamide in the medium is 35 mg. per cent). C and II are significantly higher than I.

We have studied also the effect of carbutamide on the glucose uptake of diaphragms isolated from alloxanized rats. The rats were moderately to severely diabetic and were studied 2 to 30 days after the injection of alloxan.¹⁹

In these experiments we obtained a rise in the glucose uptake of the same order of magnitude as in normal rats, without any change in the glycogen content (FIGURE 7).

Of course, these results are in disagreement with the hypothesis that the action of the oral antidiabetic compounds require the presence of beta cells capable of increasing the output of insulin. However it is not possible to prove that an alloxanized animal is an insulin-free organism, and we are well aware of the fact that our results with alloxanized rats do not disprove the hypothesis, supported by many other kinds of evidence, that the action of these compounds requires the presence of insulin. We do believe that it is important to distinguish the hypothesis of action by increased insulin secretion from the hypothesis of insulin as a permissive agent for the action of the oral antidiabetic compounds.

There is good evidence from several sources²⁰⁻²² that the oral antidiabetic compounds reduce the output of glucose from the liver, but it is known that they are active even in the absence of this organ.^{18, 23} The results presented here suggest that an action on the peripheral uptake of glucose might also contribute to the fall of blood sugar observed after administration of these compounds.

Discussion

We did not measure oxygen uptake in these experiments, but I do not think that lack of oxygen can explain the increase of glucose uptake observed in our experiments with oral antidiabetic compounds. We are working with a bicarbonate-phosphate buffer and an atmosphere of carbogen. In our control experiments with nonhypoglycemic sulfonylureas we did not find any increase in glucose uptake. Our system is highly sensitive to insulin, both on glucose uptake and on glycogen deposition.

There is some evidence of a more general enzyme inhibition with very high concentrations of sulfonylureas. A lack of increase of glucose uptake by the isolated diaphragm with such high concentrations has also been reported from other laboratories.

Summary

In clinical studies of the oral and intravenous glucose tolerance an increase of glucose tolerance was observed during the administration of carbutamide, tolbutamide, or chlorpropamide to diabetic patients. Serum inorganic phosphate was unchanged during the action of carbutamide and tolbutamide. With chlorpropamide a slight decrease in serum inorganic phosphate and in serum potassium occurred.

Histological studies of the pancreatic islets of the rat showed no changes in the alpha or beta cells during the administration of low or high doses of PTD, carbutamide, or tolbutamide for short or long periods of time.

In vitro studies of the isolated rat diaphragm showed a rise in the glucose uptake when carbutamide was injected previously; a similar rise was observed on the addition of IPTD, carbutamide, or tolbutamide to the medium *in vitro*. No change in the glycogen content of the diaphragm was observed. The same results were obtained using diaphragms from alloxanized rats with moderate or severe diabetes. Phenethylidiguamide caused a rise in

the glucose uptake and a fall in the glycogen content of the isolated rat diaphragm.

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THE PHARMACOLOGY OF CHLORPROPAMIDE

Jurg A. Schneider, Ernesto D. Salgado, Doris Jaeger, Charles Delahunt

Department of Macrobiology Research, Pfizer Therapeutic Institute, Maywood, N. J.

Ever since it was recognized that insulin could not be used orally in the treatment of diabetes mellitus, both synthetic compounds and natural products have been investigated for hypoglycemic activity pharmacologically and clinically. Severe side effects and/or insufficient activity made many of the previous attempts futile or impractical.

The recent extensive studies of certain sulfonylureas as orally active hypoglycemic agents^{1, 2} are the result of a concentrated research effort of the last sixteen years.³ When the therapeutic value of the sulfonylureas was recognized, the search for the most effective compounds of the series was intensified. Chlorpropamide was selected as a result of an extensive screening program. Its high degree of hypoglycemic activity and prolonged duration of action as evidenced by animal experimentation warranted thorough pharmacological and clinical studies.⁴

This paper summarizes the present status of pharmacological investigation with chlorpropamide, taking into account previous reports by Salgado *et al.*⁵ and Root.⁶ Emphasis is placed on new aspects; data corroborating earlier findings are referred to briefly.

Materials and Methods

The results reported were established in mice (Webster strain), rats (Wistar strain), cats, dogs, and rhesus monkeys. Routine pharmacological and biochemical methods were employed unless otherwise indicated.

All blood sugar determinations were performed according to the method of Folin and Malmros.⁷ The animals were fasted for 18 hours previous to the withdrawal of blood samples. Liver and muscle glycogen were determined by the method of Seifter.⁸ Chlorpropamide blood levels were evaluated by a spectrophotometric method developed by Toolan and Wagner.⁹

Chlorpropamide was administered orally in most experiments. Where parenteral administration was employed, the sodium salt of chlorpropamide was used (solubility in water > 50 per cent; pH of 1 per cent solution = 8.5). Radioactive material consisted of S³⁵-labeled chlorpropamide.

Results

Hypoglycemic activity of chlorpropamide. As reported previously,^{5, 6} chlorpropamide was found to cause a prolonged hypoglycemic effect in both rats and dogs. Single oral doses of 25 mg./kg. or higher were required to obtain a definite effect. Calculated on a mg./kg. basis, this is at least twice the recommended average human dose (7 to 10 mg./kg./day). When compared to tolbutamide in these two species, chlorpropamide was somewhat slower in its onset, but more prolonged in its duration of action; the maximal hypoglycemic effect of both compounds was of approximately equal magnitude.

Root⁶ had observed that, using both duration of action and degree of hypoglycemia as criteria for evaluation, in both rats and dogs chlorpropamide was more active on a weight basis than tolbutamide. The hypoglycemic effects of chlorpropamide and tolbutamide were also compared in rhesus monkeys following single oral administration. The results of such an experiment are shown in FIGURE 1. At dose levels of 10 mg./kg. orally a definite hypoglycemic response was obtained that was slower in onset, but considerably greater in magnitude and more prolonged in its duration of

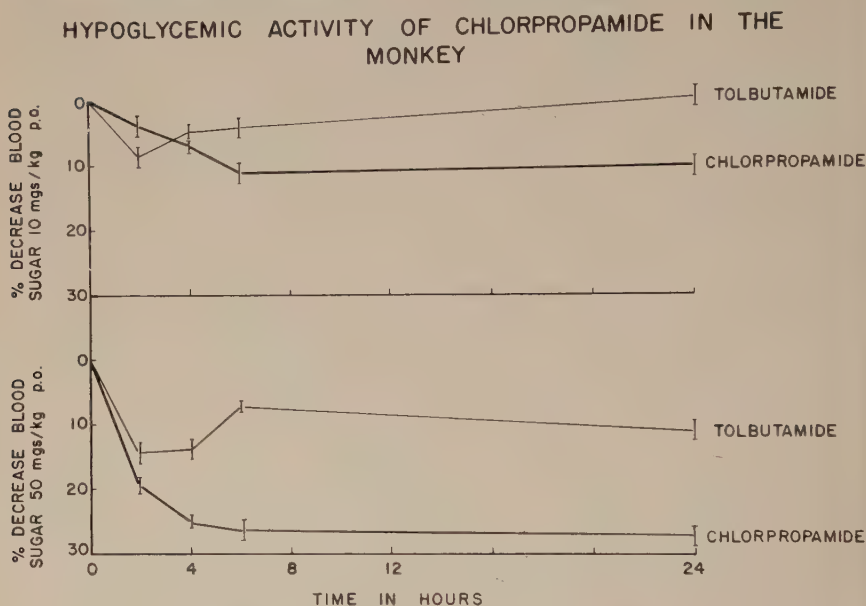


FIGURE 1. *Top tracing:* crossover studies in 4 monkeys illustrating the hypoglycemic effect of chlorpropamide as compared to tolbutamide. Each point represents the average of 8 blood-sugar determinations with their standard errors. Both drugs were given at 10 mg./kg. orally, which corresponds to the dose of chlorpropamide used clinically in human beings. *Bottom tracing:* corresponding experiment at 50 mg./kg. orally. Each point represents the average of 4 blood-sugar determinations; no crossover studies were performed. The graph illustrates the more prolonged and more pronounced hypoglycemic effect of chlorpropamide over tolbutamide in this species.

action than with tolbutamide. At doses of 5 mg./kg. orally (not shown on FIGURE 1) a definite blood sugar lowering effect was observed with chlorpropamide, whereas tolbutamide caused a slight and rather transient rise of blood sugar levels at that dose. At 50 mg./kg., which is an excessive dose, both compounds caused pronounced hypoglycemia, chlorpropamide again being more active and more prolonged in its effect than tolbutamide. Therefore, in the rhesus monkey chlorpropamide was active in dose levels that compare well to those used in the human being.¹⁰ The results also indicate that rhesus monkeys, much like human beings, are more susceptible to chlorpropamide than either dogs or rats.

In view of the long duration of action of chlorpropamide, the question arose whether chlorpropamide had cumulative effects. To clarify this point, groups of four dogs were given doses of 5, 25, and 50 mg./kg. of chlorpropamide orally for several weeks. Blood sugar and chlorpropamide blood levels were determined at regular intervals. The results of this study are shown in FIGURE 2. It can be concluded that continued administration of subthreshold doses of chlorpropamide did not result in a hypoglycemic effect, even after 2 weeks of daily medication. However larger doses caused a gradual decrease of the blood sugar that was paralleled by a rise in serum

CHRONIC ADMINISTRATION OF CHLORPROPAMIDE TO DOGS

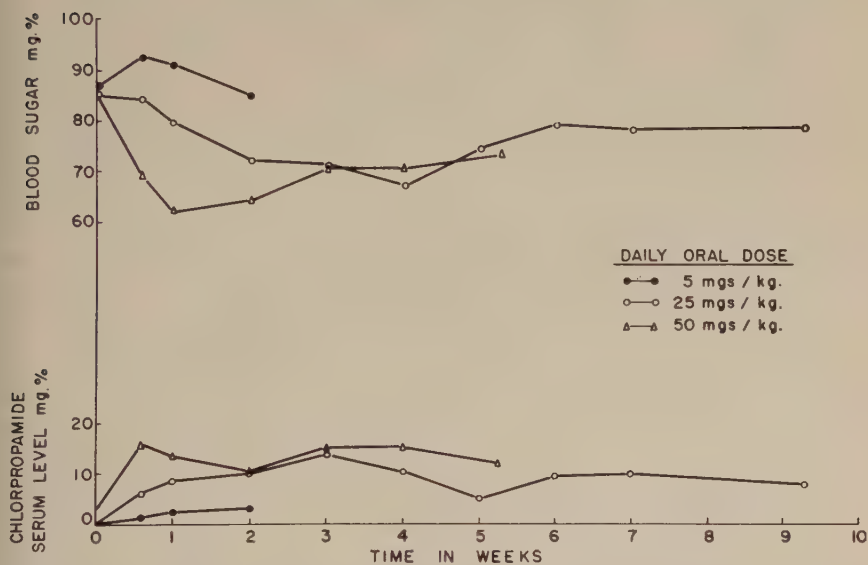


FIGURE 2. Comparison of hypoglycemic activity and blood levels of chlorpropamide in groups of 4 dogs following daily oral administration at various dose levels. Each point represents an average of 4 values. All blood sugar determinations and chlorpropamide blood levels were made 24 hours following the last dose; the animals were in a fasting state.

concentration of chlorpropamide. After several days the blood levels of chlorpropamide stabilized and did not increase beyond a certain point. As noted by Root,⁶ hypoglycemic effect and drug concentration in serum were not always in good correlation. Reduction or cessation of medication resulted in gradual reduction of chlorpropamide blood levels and return of blood sugar to normal values.

Since the method of Toolan and Wagner,⁹ is not applicable for determination of chlorpropamide in urine, excretion studies were performed in dogs with radioactive S³⁵-labeled chlorpropamide given intravenously. The results available are summarized in FIGURE 3. According to these data the radioactive material is excreted very slowly, only about 45 to 50 per cent of the material given being accounted for after 72 hours. Chromatographic

studies are in progress to determine whether chlorpropamide is excreted unchanged in the dog or whether it is metabolized.

Toxicology. In acute oral, intravenous, and intraperitoneal toxicity studies in mice, rats, and dogs, chlorpropamide was slightly more toxic than tolbutamide on a weight basis (TABLE 1); the species used, the age, and general status of the animal were important factors for survival following single doses. This finding is in accord with the more potent hypoglycemic activity of chlorpropamide over tolbutamide.

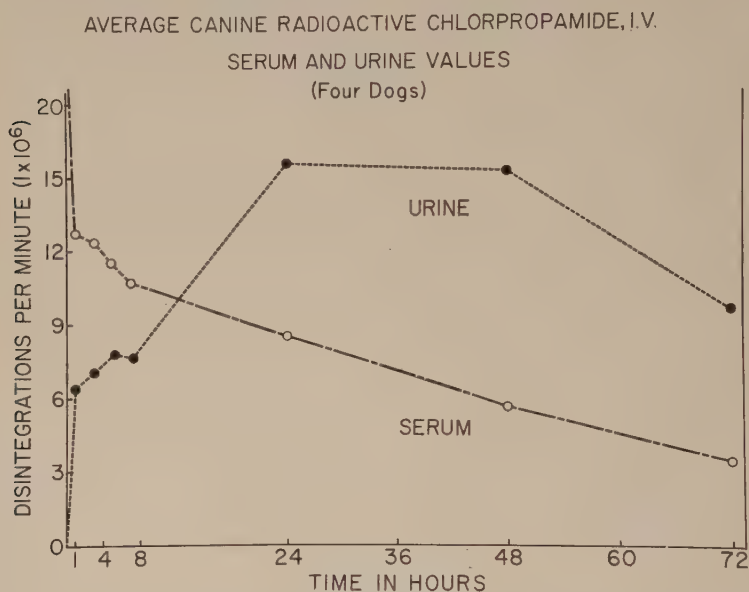


FIGURE 3. Serum levels and urinary excretion pattern of S^{35} -labeled chlorpropamide in the dog. Serum and urine samples were taken at 1, 3, 5, 7, 24, 48, and 72 hours following intravenous drug administration (50 mg./kg. chlorpropamide representing 1.5×10^8 disintegrations/min.). The samples were counted and the values plotted against time and adjusted to total blood and urine volume. The graph indicates relatively slow excretion of radioactive material.

Chronic toxicity experiments were performed in dogs, rats, and monkeys at various dose levels for periods ranging from 6 to 20 months. Dogs tolerated daily oral doses of 150 mg./kg., which corresponds to more than 20 times the recommended clinical dose in the human being. Except for occasional symptoms of ataxia and muscular weakness in some of the animals, good physical condition was maintained throughout the study. Blood sugars were low in these animals. Higher doses proved lethal following repeated administration. Toxic symptoms observed were ataxia, vomiting, diarrhea, tremors, muscular weakness, coma, and death.

Rats survived daily doses up to 500 mg./kg. in their diet for 6 months to 1 year. A most interesting phenomenon developed in this species only at the high dose levels (FIGURE 4). Chlorpropamide caused a retardation in

TABLE 1
ACUTE TOXICITY OF CHLORPROPAMIDE AND TOLBUTAMIDE

Drug	Species	Route	LD ₅₀	95% Confidence limits* mg./kg.
Chlorpropamide.....	Mice	Oral	1675	1444-1943
Chlorpropamide.....	Mice	S.C.	780	710-856
Chlorpropamide.....	Mice	I.P.	760	693-834
Chlorpropamide.....	Mice	I.V.	500	431-580
Chlorpropamide.....	Rats	Oral	2390	1943-2940
Chlorpropamide.....	Rats	S.C.	760	603-958
Chlorpropamide.....	Rats	I.P.	575	454-725
Chlorpropamide.....	Rats	I.V.	590	486-714
Chlorpropamide.....	Dogs	Oral	approx. 800	
Chlorpropamide.....	Dogs	I.V.	approx. 575	
Tolbutamide.....	Mice	Oral	1830	1591-2105
Tolbutamide.....	Mice	I.V.	700	669-731
Tolbutamide.....	Rats	Oral	2490	2029-2936
Tolbutamide.....	Rats	I.V.	775	731-817

* Whenever possible, LD₅₀ and 95 per cent confidence limits were calculated according to the method of Litchfield and Wilcoxon. CMC (carboxymethylcellulose) was employed as a suspending agent. Rats and mice used were weanlings.

THE EFFECT OF CHLORPROPAMIDE ON THE GROWTH RATE AND FOOD CONSUMPTION OF MALE ALBINO RATS (SERIES II)

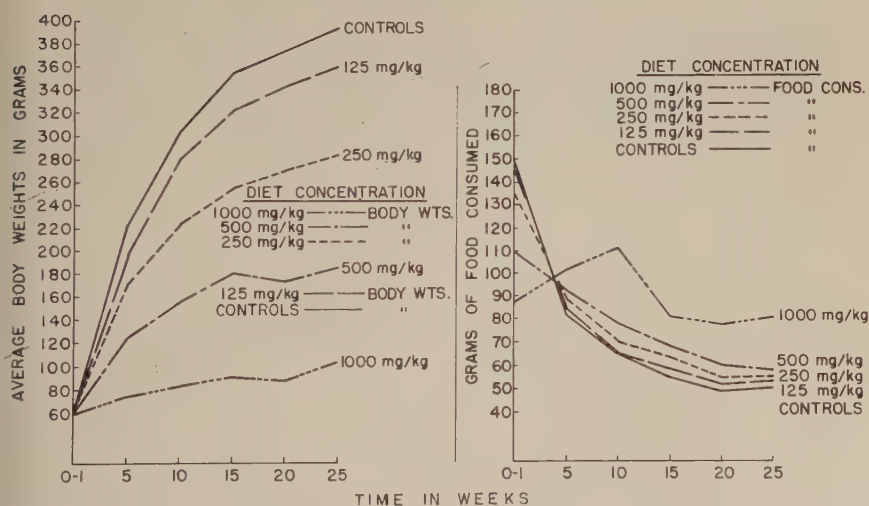


FIGURE 4. Growth-depressant effects of chlorpropamide in rats as evidenced by plotting average body weights of groups of twenty animals versus time (left graph). A dose response effect could be established clearly. Feed consumption (gm./kg.) in the corresponding groups (right graph) indicates adequate food intake.

growth that was dose-dependent; the food intake of these rats, however, did not decrease proportionally. In order to check whether chlorpropamide interfered with the availability of certain amino acids essential for growth, cysteine and/or glycine were added to the diet in equimolar concentration to chlorpropamide. Although glycine, but not cysteine, alleviated growth depression slightly, these dietary adjustments failed to overcome chlorpropamide-induced growth retardation.

Rhesus monkeys are presently being maintained on chlorpropamide orally in doses up to 200 mg./kg. (about 30 times the blood sugar lowering dose); intermittent diarrhea in the high dose range is the only toxic symptom observable.

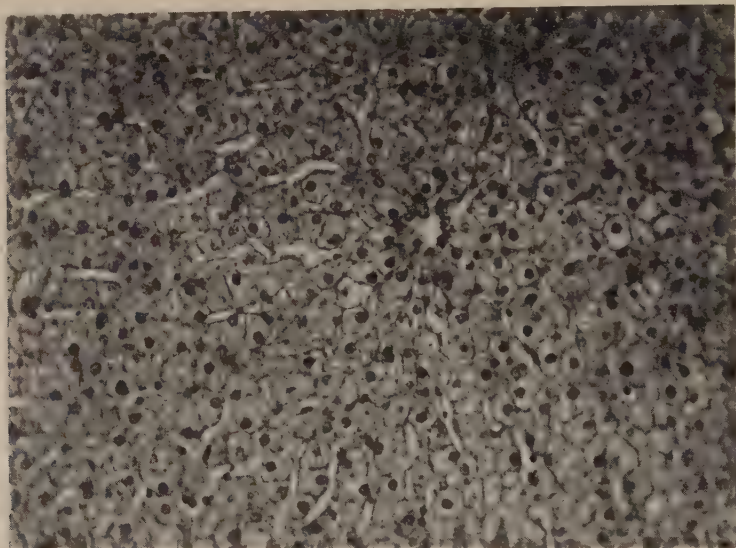


FIGURE 5. Histological section of a liver, hematoxylin-eosin stain, from a dog treated with chlorpropamide for 20 months daily at a dose of 100 mg./kg. orally. No signs of liver pathology can be observed.

Autopsies were performed on all animals sacrificed after periods of up to 20 months of continuous medication, and special attention was given to the liver. FIGURE 5 is a histological slide of a liver from a dog treated for 20 months with 100 mg./kg. of chlorpropamide orally daily. As in all other cases investigated, the liver showed no histopathological changes. This is in accord with the unimpaired liver function of these animals as evidenced by periodical liver function tests such as for alkaline phosphatase, thymol turbidity, or Bromsulphalein (BSP).

Mode of action. Despite thorough pharmacological investigation, qualitative differences between the mode of action of chlorpropamide and tolbutamide could not be established. Chlorpropamide showed a marked effect on glycogen metabolism as evidenced by increased glycogen content of the liver (FIGURE 6); on the other hand, glycogen content of striated muscle was

unaltered. Insulin sensitivity was not increased by chlorpropamide (FIGURE 7), while glucose tolerance remained within the limits of normal. In our laboratories similar results were observed with tolbutamide. In accord with the theory that sulfonylureas act, at least in part, through release of insulin, direct evidence of degranulation of the beta cells in the islets of Langerhans was obtained in rats following oral administration of chlorpropamide for several days (FIGURE 8). The phenomenon was reversible after discontinuation of medication for 72 hours. Furthermore, alloxan-diabetic rats showed no hypoglycemic reaction to chlorpropamide, whereas severe hypoglycemia

**EFFECT OF CHLORPROPAMIDE 100 mgs/kg.
p. o. ON LIVER AND MUSCLE GLYCOGEN
2 HOURS AFTER TREATMENT**

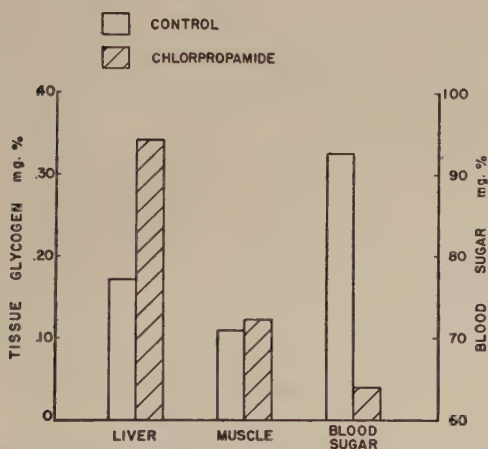


FIGURE 6. Glycogen contents in liver and muscle before and following chlorpropamide treatment in rats. Six rats were used for each determination at a dose level of 100 mg./kg. orally. The animals were sacrificed 2 hours after treatment; the blood sugar determinations taken simultaneously indicate the effectiveness of the medication. The animals were fasted throughout the test. Chlorpropamide caused an increase in liver glycogen over the control, but caused no change in muscle glycogen. This is in accord with data in the literature obtained with other sulfonylureas.

and death followed drug treatment of adrenalectomized rats (FIGURE 9). Similar experimental data are reported for tolbutamide.^{1, 2}

Chlorpropamide was also investigated thoroughly for pharmacodynamic actions other than those related to its hypoglycemic activity. It lacked specific blood pressure, respiratory, or diuretic effects in doses that caused profound hypoglycemia. There was no antagonism to histamine or acetylcholine in the isolated guinea pig ileum.

The most obvious symptoms following high doses of chlorpropamide in mice, rats, cats, and dogs are general weakness, associated with ataxia, and incoordination. They may occur within a few minutes after intravenous

administration of high doses to an unanesthetized animal. Patients receiving an overdose of chlorpropamide are known to complain of muscular weakness.¹⁰ Hypoglycemia may be a factor involved, but glucose infusions were sometimes not adequate to alleviate these symptoms. On the other hand, the possibility of a direct pharmacological effect of chlorpropamide on electro-

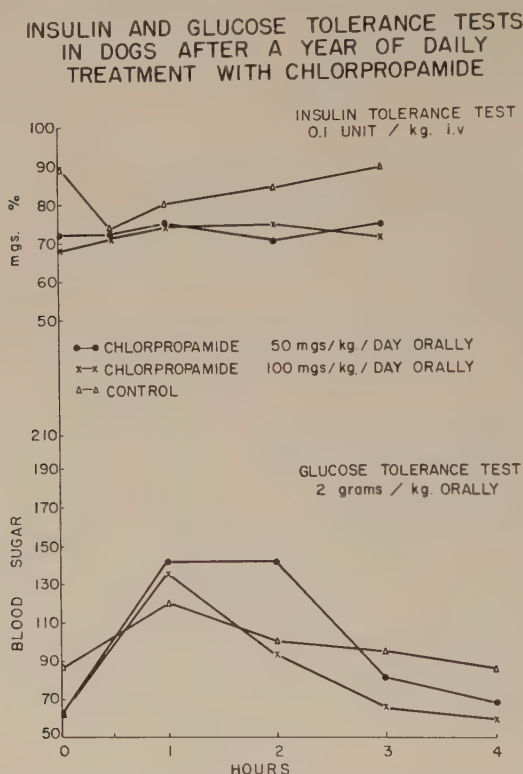


FIGURE 7. Insulin sensitivity and glucose tolerance tested in dogs following daily oral treatment with chlorpropamide for one year. *Top tracing*: insulin 0.1 unit/kg. I.V. caused a drop in blood sugar in control animals; animals receiving chlorpropamide did not react to insulin. *Bottom tracing*: glucose tolerance tests in these same animals showed a greater rise of the blood sugar concentration following one year's treatment. However the response was still in the normal range for dogs; furthermore, recovery was complete after four hours.

lyte balance, muscular function, spinal reflexes, or central nervous system must be considered.

The electrolyte balance was studied in dogs who received repeated sublethal doses of chlorpropamide until a state of prostration was obtained. Sodium and potassium values in serum were within limits of normal, with few exceptions (TABLE 2). On the other hand, it is noteworthy that these dogs appeared to respond more rapidly and more completely to combined infusions of glucose and isotonic saline solutions than to glucose alone.

TABLE 2

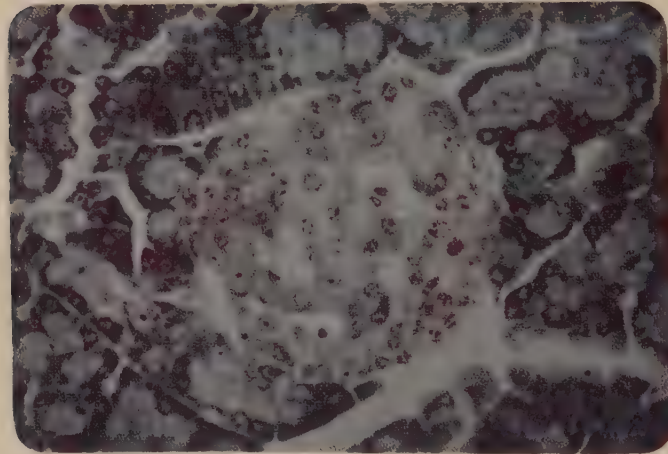
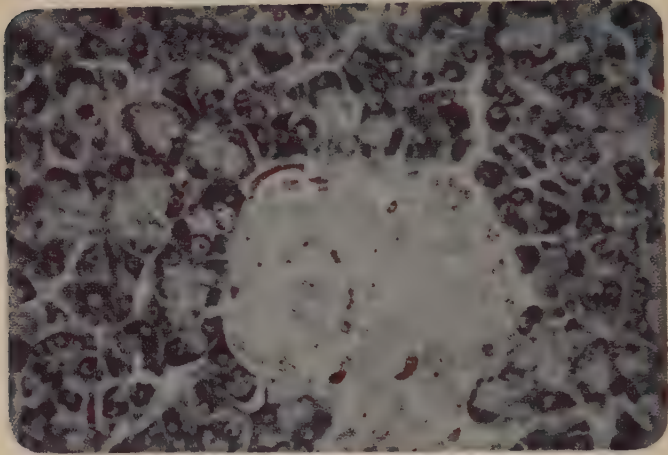
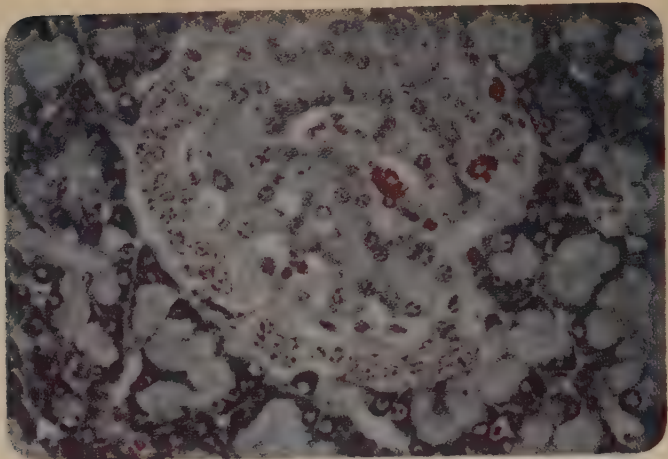
SERUM Na⁺, SERUM K⁺ (mEq./l.) VALUES OF FEMALE MONGREL DOGS RECEIVING DAILY ORAL DOSES OF CHLORPROPAMIDE

(Sample Taken 2 Hours Post Dose Unless Otherwise Stated)

Dog No.	Daily dosage chlor-propamide mg./kg.	Prechlor-propamide		Post Rx† Shown		Post Rx Shown		Post Rx Shown		Post Rx Shown		Post Rx Shown		
		Na ⁺	K ⁺	Na ⁺	K ⁺	Na ⁺	K ⁺	Na ⁺	K ⁺	Na ⁺	K ⁺	Na ⁺	K ⁺	
3159	150 6×			Rx 5		Rx 13		Rx 21		Rx 34		Rx 49		Severe toxic symptoms after Rx 49.
	200 16×	156.3	5.63	146.2	5.28	147.5	5.30	142.4	4.48	145.5	3.64	168.1	6.11	
	300 27×													
3171	150 6×	not read	6.90*	Rx 5		Rx 13		Rx 21		Rx 35		5 hr. post Rx 43		(Values at onset of severe toxic symptoms.)
	200 16×			152.0	5.83	144.1	5.20	145.9	4.67	155.2	4.22	141.7	6.25	
	300 21×													
3163	150 6×			Rx 5		Rx 13		Rx 21		24 hr. post Rx 34		(Values at onset of severe toxic symptoms.)		
	200 16×	147.2	5.34	150.0	5.74	145.1	5.25	140.8	4.44	147.7	4.34			
	300 12×													
3162	150 6×			Rx 5		Rx 13		Rx 21		Rx 35		Rx 38		Died 24 hours after Rx 38, showing severe toxic symptoms.
	200 16×	161.4	7.78*	156.2	5.64	139.1	6.67	143.9	3.96	147.7	4.03	115.7	4.86	
	300 16×													
2953	150 6×			Rx 5		Rx 13		Rx 21		Rx 27		Died overnight after Rx 28. Severe toxic symptoms not observed.		
	200 16×	151.3	5.09	155.4	5.54	153.7	5.15	134.2	6.52	150.0	5.00			
	300 5×													
	400 1×													
3166	150 3×			Rx 4		Rx 12		Rx 19		Rx 26		Died overnight after Rx 26. Severe toxic symptoms not observed.		
	200 10×	154.9	5.71	145.1	5.00	140.8	5.19	146.5	2.94	150.0	4.80			
	300 13×													
3023	150 6×			Rx 5		Rx 13		Rx 21		6 hr. post Rx 23		(Values at onset of severe toxic symptoms.)		
	200 16×	146.7	5.39	153.4	5.25	148.5	5.00	136.7	4.58	144.8	3.66			
	300 1×													
3173	150 4×			Rx 4		Rx 12		Rx 15		Died after Rx 16 showing severe toxic symptoms.				
	200 9×	154.9	4.68	140.1	6.36	131.1	4.48	143.8	4.86					
	300 3×													
3160	150 6×			Rx 5		Rx 9		Died overnight after Rx 10. Severe toxic symptoms not observed.						
	200 4×	138.7	5.90	159.3	5.05	140.3	6.32							
3009	150 6×			Rx 5		Rx 9		Severe toxic symptoms after Rx 10.						
	200 4×	158.7	6.40*	154.0	5.58	97.8	5.77							
3108	150 3×			Rx 4		24 hr. post Rx 7		(Values at onset of severe toxic symptoms.)						
	200 4×	157.4	5.44	137.6	6.9	145.9	5.78							
3000	150 3×			Rx 4		Rx 7		Severe toxic symptoms after Rx 7.						
	200 4×	149.0	5.59	136.13	5.86	141.2	4.79							

* These serum K⁺ values that are higher than normal for the dog (3.7 to 6 mEq./l.) may reflect the fact that these dogs were relatively recent arrivals in the laboratory and their feeding patterns had not yet stabilized.

† Rx represents number of doses following which blood was withdrawn for analysis.



In the cat nerve muscle preparation there was evidence of a direct effect of high doses of chlorpropamide on contractile force (FIGURE 10). Mono-synaptic (knee jerk) and polysynaptic spinal reflexes (flexor reflex) in cats were decreased gradually; however, all these effects were not sufficiently marked to explain the degree of weakness observed in the intact animals.

**HYPOGLYCEMIC EFFECT OF A SINGLE
ORAL DOSE OF CHLORPROPAMIDE
100 mg/kg IN RATS**

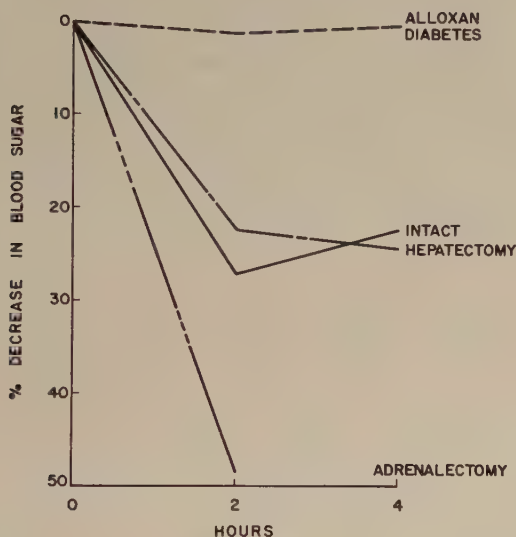


FIGURE 9. The effect of single oral doses of 100 mg./kg. chlorpropamide to rats under various conditions. Blood sugar determinations were made every 2 hours. Fasting conditions were maintained throughout the test. Six rats were used in each group, and the blood sugar responses of the groups were compared at each point with the preinjection level. Alloxan-diabetic rats showed no hypoglycemia; adrenalectomized animals responded with a large drop in blood sugar followed by death between 2 and 4 hours after drug administration. No significant difference was observed in hepatectomized and intact animals.

Electroencephalographic (EEG) studies performed in cats immobilized with succinylcholine showed no significant change in doses of as much as 50 mg./kg. I.V. Arousal reactions evoked by acoustic or sciatic stimulation were not abolished. Only after doses of 150 mg./kg. I.V. or more was a slowing of the EEG noted; spindles were observed occasionally, but incon-

FIGURE 8. Effect of chlorpropamide on beta cell granules of rat pancreas stained with Gomori's chrome-alum-hematoxylin. *Top*: section of normal pancreas showing distinct granulation of beta cells. *Center*: degranulation of beta cells of rat pancreas after treatment with chlorpropamide (300 mg./kg.) orally for 5 days. Note the lack of granulation. *Bottom*: Complete recovery indistinguishable from control was observed in animals following cessation of medication for 72 hours.

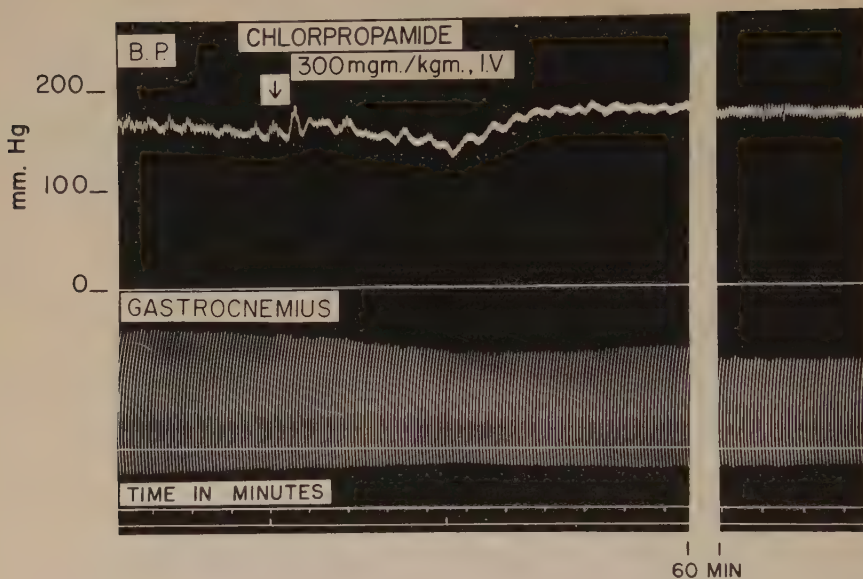


FIGURE 10. The effect of high doses of chlorpropamide on contractile force of the cat sciatic nerve gastrocnemius preparation (Nembutal anesthesia). The muscular contraction was reduced only by about 20 per cent following intravenous administration of high doses of chlorpropamide.

sistently. During these experiments, the blood pressure varied from 110 to 130 mm. Hg, and showed only a slight rise after drug administration.

Discussion

Although the hypoglycemic effect of chlorpropamide could be demonstrated in rats, rabbits, and dogs, the rhesus monkey was not only most sensitive to the drug but, like the human being, showed a more pronounced and more prolonged hypoglycemic response than it did to tolbutamide. The metabolic fate of chlorpropamide may vary from species to species and may explain these quantitative differences. Such species variations in metabolism for tolbutamide are known to exist between dogs and human beings.¹¹

A cumulative effect may occur in dogs for the first few days following oral administration at various dose levels, but an equilibrium was reached within a few days beyond which cumulation did not progress. As observed by Root,⁶ individual dogs showed little correlation between dosage and serum concentration of chlorpropamide. However, the average figures indicated a definite dose response effect. This is in accord with studies of Carlozzi *et al.*¹² in the human being.

From the limited data available on the fate and excretion of chlorpropamide in dogs only, few conclusions can be drawn at this time. The long duration of action of chlorpropamide appears to parallel a slow rate of excretion.

Chromatographic analysis of urine will determine whether chlorpropamide

is metabolized in the dog; unchanged excretion is reported for the human being.¹³ The difference in hypoglycemic potency between dogs, on the one hand, and human beings and monkeys, on the other, may depend upon differences in metabolism. It has been suggested⁴ that chlorination of the phenolic group prevented carboxylation of the compound in the para position, a reaction known to take place with tolbutamide in the human being.¹¹

When given chronically to rats, dogs, and monkeys, chlorpropamide was tolerated without untoward side effects in doses at least twenty times the recommended human dose. This represents a remarkable degree of safety for a powerful hypoglycemic agent. Of special interest was the lack of histopathological changes in livers of rats and dogs treated with high doses of chlorpropamide for periods of up to twenty months. These results are in agreement with findings of Root,⁶ and they support the view that reversible jaundice, encountered only very rarely in human patients, is more a matter of individual idiosyncrasy than a toxic effect characteristic for chlorpropamide. It may be necessary to consider such factors as history of liver disease, impaired liver function prior to medication, or even increase in biliary secretion as described by Purnell *et al.*¹⁴ for the sulfonyleureas.

Growth inhibition as seen exclusively in the rat experiments may have various causes and may eventually offer an approach to the mode of action of chlorpropamide. However, the results available at present do not allow an analysis of this drug effect. Metabolic interference of chlorpropamide with cysteine and/or glycine supply to the growing organism was considered, but this reaction is unlikely because addition of these amino acids to the diet did not overcome growth depression. It should be remembered that nonspecific stress¹⁵ might be a factor.

The investigation of some of the toxic effects of chlorpropamide is of value in view of the nature of certain side effects, such as muscle weakness, observed in human beings following overdosage. Obviously, ataxia and atonia are not necessarily related to hypoglycemia, but to the drug itself. Potassium loss does not seem to be a factor, as evidenced by normal serum potassium values in dogs and by normal muscle potassium concentration in rats treated with high doses of chlorpropamide.¹⁶ The empirical fact that dogs treated with high doses of chlorpropamide responded better to combined glucose-sodium infusions than to glucose alone is yet unexplained, as no evidence of sodium deficiency could be obtained in these animals.

In itself the direct effect of chlorpropamide on the contractile force of striated muscle was not definite enough to account for ataxia and weakness. The same was true for the reflex depression obtained in cats.

Gerstenberg *et al.*¹⁷ had observed reflex depression in rabbits with tolbutamide, an effect that seemed to be much more pronounced and was assumed to be unrelated to hypoglycemia.

It is doubtful whether the EEG findings following high single doses of chlorpropamide offer an explanation for the side effects observed with toxic doses of the drug. Amounts of chlorpropamide necessary to elicit these EEG changes must be considered excessive. However, it is conceivable that hypoglycemia as well as central and peripheral nervous system effects of

chlorpropamide are contributing to the symptoms described for the intact organism.

Summary

Chlorpropamide is a potent orally active hypoglycemic agent of long duration of action. Its activity may be demonstrated best in rhesus monkeys where it shows activity in the dose range used in human beings. Fate studies in dogs indicate slow excretion of the compound in acute experiments.

Upon chronic administration to various species of animals, chlorpropamide was found to be well tolerated in doses twenty times greater than the recommended human dose. With the exception of growth retardation in very high doses in rats, the compound produced no gross anatomical or histopathological changes.

Chlorpropamide is qualitatively similar to, but quantitatively more potent than, other hypoglycemic agents of the sulfonylurea types. Evidence was presented that certain central nervous system effects observed experimentally after high doses, or clinically following overdosage, may be related to a direct action of the drug rather than to its hypoglycemic effect.

Acknowledgments

The work reported and summarized in this paper contains data obtained from studies performed by S. Y. P'an, J. Kaiser, M. Finkelstein, K. Finger, J. F. Snell, L. Schorr, A. Drakontides, and their staff, whose efforts are greatly appreciated.

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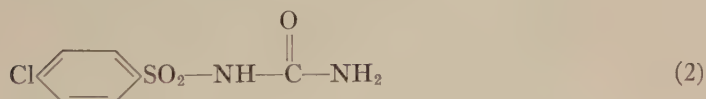
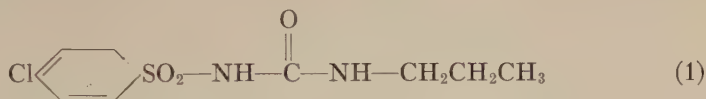
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Discussion of the Paper

MARY A. ROOT (*The Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Ind.*): Jurg A. Schneider and I are apparently in complete agreement about the pharmacology of chlorpropamide.¹ However, I can add a bit to his study of its metabolism. All of this study of metabolism has been done by John Welles of our Toxicology Department.

Schneider mentioned that paper chromatography of urine from dogs given S³⁵-labeled chlorpropamide yielded three spots. We have used unlabeled chlorpropamide in similar experiments and have also found three spots from dog urine. The spots were eluted from the paper, crystallized and then identified by x-ray diffraction and infrared studies. The formulas of these three substances are:



The total amount of each metabolite was determined by elution from the paper and measurement of the ultraviolet absorption on a recording spectrophotometer. This method is not completely quantitative, but does give approximate values.

In normal dogs maintained on a constant daily dose of chlorpropamide, from 27 to 33 per cent of the drug (Formula 1) was excreted unchanged every 24 hours. The sulfonylurea (Formula 2) accounted for from 35 to 40 per cent of the administered dose, and the sulfonamide (Formula 3) for another 16 to 24 per cent. This gives a total 24-hour recovery of from 78 to 97 per cent of the daily dose. These studies do not exclude the possibility that there are other metabolites of chlorpropamide, but Welles has been able to account for the major portion of the drug.

The metabolic products of both carbutamide and tolbutamide have been

shown to vary greatly from species to species.²⁻⁴ For this reason, we have investigated the major metabolic products of chlorpropamide in rabbits. In these animals, 80 to 95 per cent of a dose of chlorpropamide was excreted unchanged (Formula 1). Preliminary studies in man indicate that some of the drug is excreted unchanged but that there are probably 1 or 2 metabolic products. Much more work must be done before we can establish the existence of such products and, if so, their identity.

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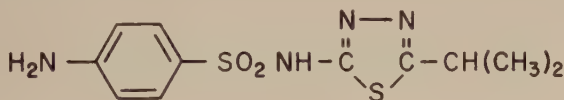
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HYPOGLYCEMIC SULFONYLUREAS: EFFECT OF STRUCTURE ON ACTIVITY

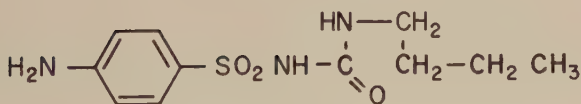
William M. McLamore, G. M. Fanelli, S. Y. P'an, G. D. Laubach

Research Laboratories, Chas. Pfizer & Co., Inc., Brooklyn, N. Y.

Although a very considerable literature has accumulated on the pharmacological and clinical properties of 2 or 3 sulfonylurea drugs, little or nothing has been published until quite recently on the effects of structural changes on hypoglycemic activity within this general class.¹⁻⁴ We thought it would be of interest, therefore, as background for this monograph, to outline the results of such a study, which has been under way in our laboratories for some time, and which has led to the hypoglycemic agent chlorpropamide (Diabinese). Moreover, although our results are in substantial agreement with those recently published, there are some minor differences.



2254 RP (I P T D)



CARBUTAMIDE (BZ-55)

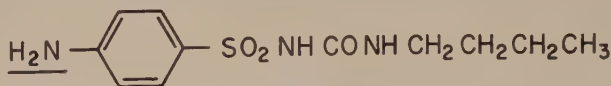
FIGURE 1

The detailed history of the hypoglycemic sulfonylureas is too well known to require repetition here. One facet of this history, however, is pertinent to the present discussion; namely, the close structural resemblance (FIGURE 1) of the early sulfanilylthiadiazoles (1942)^{5, 6} to the later sulfonylureas (1955).⁷ In view of this close similarity it is perhaps surprising that the hypoglycemic effects of sulfonylureas were discovered so much later and apparently completely by chance.

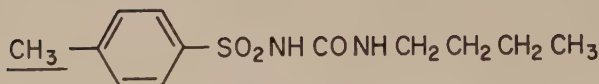
Discovery of the hypoglycemic properties of 1-butyl-3-sulfanilylurea or carbutamide⁷ (BZ 55, Invenol, Nadisan) was soon followed by the discovery^{8, 9} that the *p*-amino group (FIGURE 2) was not necessary for activity. The *p*-methyl analogue, tolbutamide⁹ or 1-butyl-3-*p*-tolylsulfonylurea (FIGURE 2), which has become well known in this country as Orinase (Upjohn), was shown to be an effective oral hypoglycemic agent. One immediately apparent

advantage of this structural change was the complete loss of antibacterial properties—a predictable result, which was carefully confirmed for tolbutamide. On the other hand, it soon became apparent that tolbutamide, while comparable to carbutamide in potency, was a shorter-acting drug.

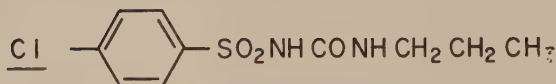
A considerable number of sulfonylureas have been synthesized in our laboratories and screened pharmacologically for their effect on the blood sugar



CARBUTAMIDE



TOLBUTAMIDE



CHLORPROPAMIDE

FIGURE 2

of fasting white rats at several intervals after oral administration of 100 to 300 mg./kg. of the compound. Our goal, of course, was to find a drug of superior potency and duration of action and with minimal side effects. The structure-activity correlations resulting from this study are outlined below.

As illustrated in FIGURE 3, the generalized sulfonylurea structure can be considered in three portions: (1) the alkyl chain, R ; (2) the urea portion; and

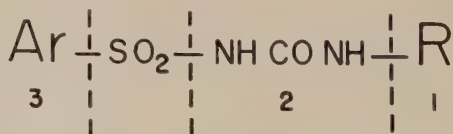


FIGURE 3

(3) the (substituted) aryl group. The effects on activity of structural changes in each of these three portions, taken one at a time, are indicated in FIGURES 4 to 8.

In most of the active series, simple alkyl chains of 3 to 4 carbon atoms led to peak activity. Homologues having a branched alkyl chain, such as *tert.*-butyl, or a cyclic alkyl substituent had quite good activity, with a probable shift of peak activity in the cyclic case to $\text{C}_5\text{--C}_7$. Compounds

Ar SO₂ NH CONH R


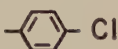
<u>R</u>	<u>ACTIVITY</u>
n-C ₃ H ₇ to n-C ₄ H ₉	+++ to ++++
BRANCHED C ₃ H ₇ to C ₅ H ₁₁ (e.g. tert-C ₄ H ₉)	++ to ++++
CYCLIC C ₅ H ₉ to C ₆ H ₁₁	+++ to ++++
NHR = N $\begin{matrix} R_1 \\ R_2 \end{matrix}$, e.g. -N 	++ to +++
R = aryl, e.g.  Cl	++ to +++

FIGURE 4. Effect of the alkyl chain (R) on activity.

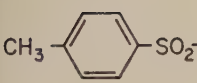
	<u>ACTIVITY</u>	<u>DURATION</u>
 <div style="display: inline-block; vertical-align: middle; margin-left: 10px;"> $\left\{ \begin{array}{l} \text{NH} - \overset{\overset{\text{S}}{\parallel}}{\underset{\underset{\text{O}}{\parallel}}{\text{C}}} - \text{NH} - \text{C}_4\text{H}_9 \\ \text{NH} - \overset{\overset{\text{O}}{\parallel}}{\underset{\underset{\text{O}}{\parallel}}{\text{C}}} - \text{O} - \text{C}_4\text{H}_9 \\ \text{NH} - \overset{\overset{\text{O}}{\parallel}}{\underset{\underset{\text{O}}{\parallel}}{\text{C}}} - \text{CH}_2 - \text{CH}_3 \\ \text{NH} - \overset{\overset{\text{O}}{\parallel}}{\underset{\underset{\text{O}}{\parallel}}{\text{C}}} - \text{CH}_2 - \text{C}_4\text{H}_9 \\ \text{NH} - \overset{\overset{\text{O}}{\parallel}}{\underset{\underset{\text{O}}{\parallel}}{\text{C}}} - \text{C}_6\text{H}_5 \\ \text{NH} - \text{NH} - \overset{\overset{\text{O}}{\parallel}}{\underset{\underset{\text{O}}{\parallel}}{\text{C}}} - \text{NH} - \text{C}_4\text{H}_9 \\ \text{NH} - \text{NH} - \overset{\overset{\text{O}}{\parallel}}{\underset{\underset{\text{O}}{\parallel}}{\text{C}}} - \text{C}_3\text{H}_7 \text{ iso} \\ \text{N}(\text{CH}_3) - \overset{\overset{\text{O}}{\parallel}}{\underset{\underset{\text{O}}{\parallel}}{\text{C}}} - \text{NH} - \text{C}_4\text{H}_9 \end{array} \right.$ </div>	<div style="display: inline-block; vertical-align: middle; margin-left: 10px;"> $\left\{ \begin{array}{l} ++ \\ + \\ \pm \\ \pm \\ + \\ \pm \\ \pm \\ \pm \end{array} \right.$ </div>	<div style="display: inline-block; vertical-align: middle; margin-left: 10px;"> $\left\{ \begin{array}{l} + \\ \\ \\ \\ + \\ \\ \\ \end{array} \right.$ </div>

FIGURE 5. Effect on activity of simple changes in the urea portion.

in which the N'-atom was disubstituted were moderately active. Replacement of the alkyl substituent by aryl also afforded some compounds of good activity.

Modifications in the urea portion of the molecule that have been studied are outlined in FIGURE 5 (simple changes) and FIGURE 6 (more complex changes). Such modifications led almost uniformly to loss of activity; weak activity was retained in a few of the structural variants, notably the thiourea, the carbamate, and one of the more complex analogues.

Variations in the aryl portion of the molecule (FIGURES 7 and 8) were the most fruitful of all the structural modifications studied. In general, *p*-substituted aryl substituents led to compounds of maximal activity and duration of action. Unsubstituted phenyl derivatives were usually less active; *o*-substituted and di-substituted (2,4; 2,5; 3,4)-phenyl compounds were still

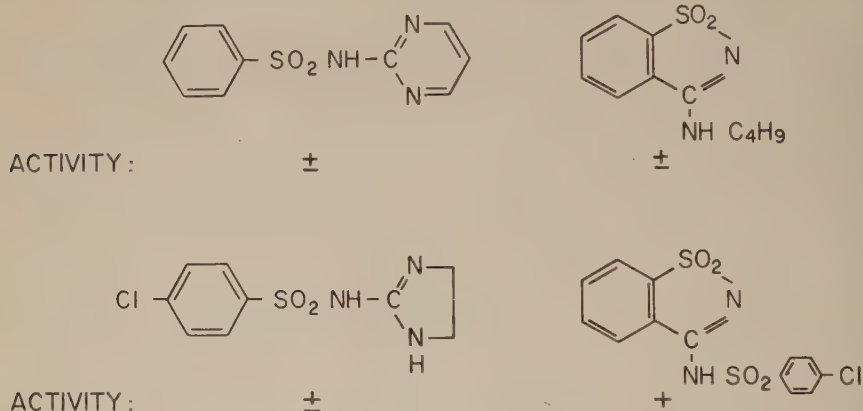


FIGURE 6. Effect on activity of more complex changes in the urea portion.

less active. Naphthylsulfonyleureas were very weakly active. Compounds in which the aryl group was heterocyclic (for example, 2-thienyl, 5-chloro-2-thienyl) were, however, moderately active.

The most effective of all *p*-substituents studied was halogen (Cl, Br), and especially chlorine. From a considerable number of *p*-chlorobenzene-sulfonyleureas with various alkyl substituents, the propyl compound was chosen on the basis of extensive animal work as the most potent and longest-acting of all the sulfonyleureas studied in our program. This compound has received the generic name chlorpropamide and the Pfizer trademark Diabinese.

Extensive further work in animals and in man has demonstrated this drug to be distinctly more potent and longer-acting in all species than previous sulfonyleureas. The relatively short duration of action of tolbutamide in man can very probably be attributed to its fairly rapid and complete metabolic oxidation to the inactive *p*-carboxy derivative (FIGURE 9). Chlorpropamide cannot, of course, be oxidatively metabolized in this manner. This fact is in accord with the observation that blood levels of chlorpropamide decrease







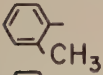
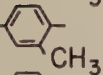

<u>Ar</u>	<u>R</u>	<u>ACTIVITY</u>	<u>DURATION</u>
Cl 	C ₃ H ₇	++++	+++
(CHLORPROPAMIDE)			
Br 	C ₃ H ₇	++++	+
F 	C ₃ H ₇	++	++
CH ₃ 	C ₄ H ₉	+++	++
(TOLBUTAMIDE)			
	C ₄ H ₉	±	
CH ₃ 	C ₃ H ₇	++	++
	C ₃ H ₇	+++	++

FIGURE 7. Effect on activity of substitution in the aryl portion.



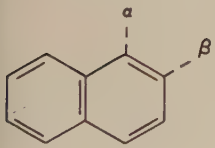
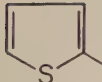
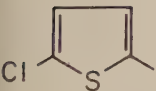
<u>Ar</u>	<u>R</u>	<u>ACTIVITY</u>	<u>DURATION</u>
	α C ₄ H ₉	±	++
	β C ₄ H ₉	+	++
	C ₄ H ₉	++	+
	C ₄ H ₉	++	+

FIGURE 8. Effect on activity of more drastic changes in the aryl portion.

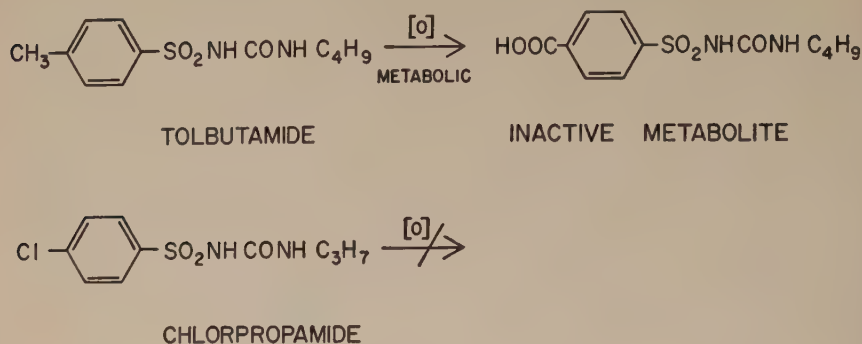


FIGURE 9

much less rapidly than those of tolbutamide, and that the drug produces hypoglycemia of longer duration.

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THE PHYSICAL PROPERTIES OF CHLORPROPAMIDE AND ITS DETERMINATION IN HUMAN SERUM

Thomas J. Toolan and Richard L. Wagner, Jr.

Chas. Pfizer & Co., Inc., Brooklyn, N. Y.

Chlorpropamide [1-(*p*-chlorobenzenesulfonyl)-3-propylurea; Diabinese*] belongs to the class of arylsulfonylurea compounds, which have found widespread use in the oral treatment of diabetes mellitus. It was felt worthwhile to study some of the physical properties of this compound and to develop a method suitable for its determination in human serum.

Chlorpropamide can be characterized by a number of physical properties. The present paper deals with preliminary observations on the following: (1) titration behavior, (2) melting point, (3) ultraviolet absorption spectrum, (4) infrared absorption spectrum, (5) solubility, (6) paper chromatography, and (7) countercurrent distribution. Such data proved useful in the development of an assay method for this product.

Several assay methods have been reported for 2 other arylsulfonylurea compounds; namely, tolbutamide and carbutamide.^{1†} Forist *et al.*,² Spingler and Kaiser,³ and Bladh and Nordén⁴ developed methods for the determination of tolbutamide (1-butyl-3-*p*-tolylsulfonylurea); these were based essentially on the intense ultraviolet absorption of this compound at 228 m μ . In addition, McDonald and Sawinski⁵ and Spingler⁶ described colorimetric procedures for the determination of tolbutamide. Carbutamide (1-butyl-3-sulfanilylurea) can be determined in serum by diazotization and coupling reactions.⁷ The method to be described for chlorpropamide is based on the ultraviolet absorption of this compound at 232.5 m μ in 0.01 N hydrochloric acid. It is isolated from serum by a two-stage extraction procedure which includes: (1) extraction of serum, acidified with phosphoric acid, with chloroform, and (2) re-extraction of the chloroform phase with a dilute solution of sodium carbonate. The aqueous phase from the second extraction is adjusted to pH 2 with hydrochloric acid; the chlorpropamide content is determined by measurement of the absorbance at 232.5 m μ .

EXPERIMENTAL

Physical Properties of Chlorpropamide

Titration. Chlorpropamide is an acid; it can be titrated as such in several solvent mixtures. Dioxane-water, acetone-water, and U.S.P. ethanol-water were found to be quite useful for this purpose; equal volumes (10 ml.) of each solvent were utilized in these mixtures. Chlorpropamide (70 mg.) was dissolved in the dioxane-water medium and was then titrated potentiometrically with standard 0.5 N sodium hydroxide. The resulting titration curve (FIGURE 1) is that of a monobasic acid. A blank titration was also run

* Diabinese is the trademark of Chas. Pfizer & Co., Inc., for chlorpropamide.

† These compounds are referred to in the literature under various designations and names (Constam¹).

on the solvent mixture alone. An equivalent weight of 279 was found; that calculated for $C_{10}H_{13}N_2O_3SCl$ is 276.7. In dioxane-water a $pH_{1/2}$ value of 6.5 was indicated for chlorpropamide; whereas the $pH_{1/2}$ values in acetone-water and U.S.P. ethanol-water are 6.2 and 6.1, respectively. The $pH_{1/2}$ value corresponds to the pH at the 50 per cent neutralization point in these titration curves. These determinations were made with a Beckman Model G pH meter.

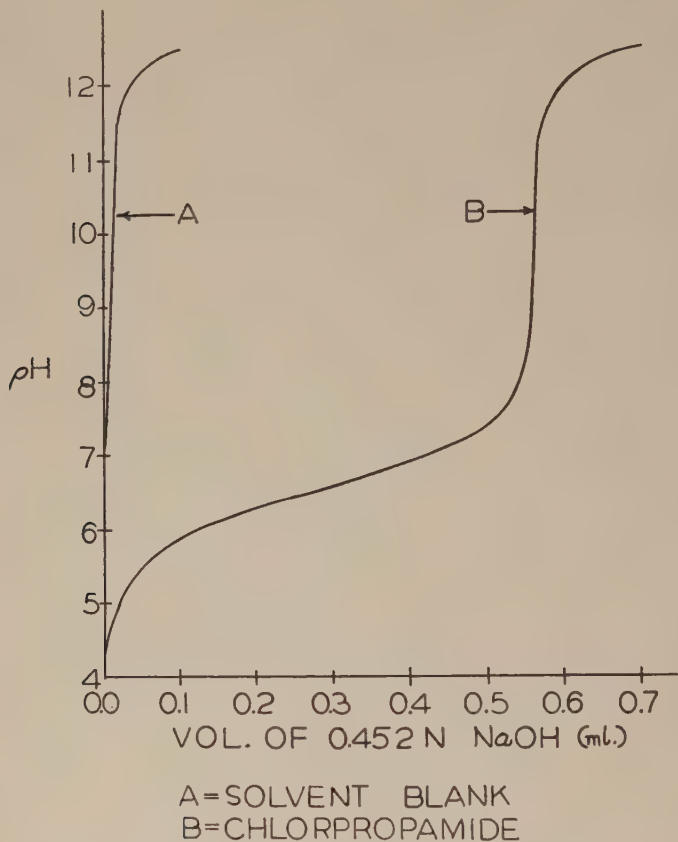


FIGURE 1. Potentiometric titration of chlorpropamide in dioxane-water.

Melting point. Chlorpropamide melts at 127.5° to 128.5° C. Previously Marshall and Sigal⁸ reported a range of 127° to 129° C, and Ruschig *et al.*,⁹ a range of 126° to 128° C.

Ultraviolet absorption spectra. In 0.01 N hydrochloric acid chlorpropamide exhibits an ultraviolet absorption maximum at $232.5\text{ m}\mu$ ($\epsilon = 16.5 \times 10^3$) and a series of less intense absorption maxima through the 250 to $280\text{ m}\mu$ wave length region. The latter occur at approximately $257.5\text{ m}\mu$, $265.5\text{ m}\mu$, $269\text{ m}\mu$ and $277\text{ m}\mu$; with $\epsilon = 710$ at $265.5\text{ m}\mu$ (FIGURE 2). Absorbance at $232.5\text{ m}\mu$ follows Beer's law over the concentration range 0–15 γ/ml .

In U.S.P. ethanol, chlorpropamide shows a maximum at $231.5\text{ m}\mu$ ($\epsilon = 15.8 \times 10^3$) and a series of weaker absorption maxima through the 250 to $280\text{ m}\mu$ wave length region. The latter occur at approximately $258\text{ m}\mu$, $265\text{ m}\mu$, $268.5\text{ m}\mu$, and $276.5\text{ m}\mu$; with $\epsilon = 580$ at $265\text{ m}\mu$.

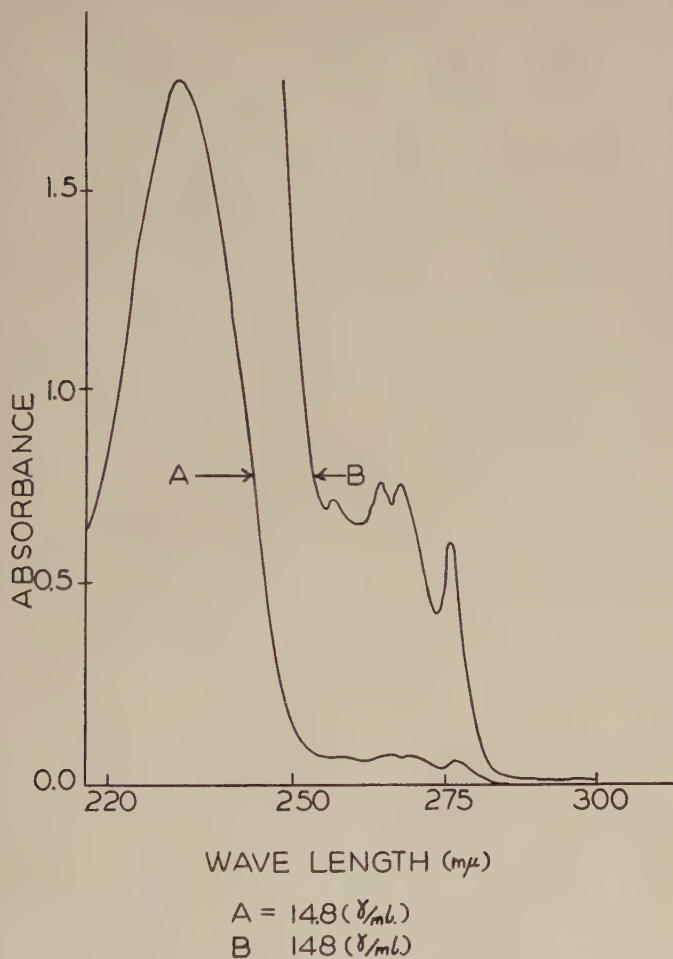


FIGURE 2. Ultraviolet absorption spectrum of chlorpropamide in 0.01 N HCl .

In 0.01 N sodium hydroxide chlorpropamide exhibits an absorption maximum at $228.5\text{ m}\mu$ ($\epsilon = 13.2 \times 10^3$); absorption in the 250 to $280\text{ m}\mu$ region is not as clearly defined as in the above two solvents.

Infrared absorption spectra. Characteristic absorption maxima in the infrared spectrum of chlorpropamide between $2\text{ }\mu$ to $15\text{ }\mu$ are shown in FIGURE 3. These spectra were determined on a Baird recording spectrometer on a Nujol mull, a potassium bromide pellet, and a chloroform solution.

Solubility. Chlorpropamide is soluble in a variety of the common organic

solvents. Concentrations of 25 mg./ml. are readily obtainable at room temperature in chloroform, acetone, ethanol, methanol, methylene chloride, ethyl acetate, isopropanol, and dioxane. The compound is less soluble than this in such solvents as benzene, carbon tetrachloride, carbon disulfide, and 2,2,4-trimethylpentane. Chlorpropamide has a limited solubility in water, particularly in acid media. The solubility of chlorpropamide in buffered (MacIlvaine) aqueous solutions at pH 2.2 to 6.0 was determined after 2 hours' equilibration at 25° C. The amount of this compound that dissolves in this pH range is as follows: pH 2.2, 0.1 to 0.2 mg./ml.; pH 3.0, 0.1 to 0.2 mg./ml.; pH 4.0, 0.1 to 0.2 mg./ml.; pH 5.0, 0.3 to 0.4 mg./ml.; pH 6.0, 2.1

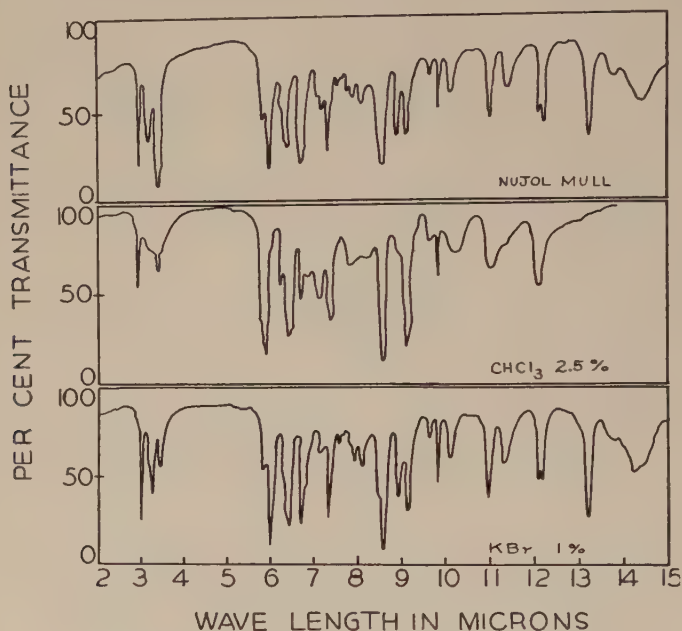


FIGURE 3. Infrared absorption spectra of chlorpropamide.

to 2.3 mg./ml. At pH values greater than 6.5 concentrations of chlorpropamide greater than 5 mg./ml. are readily obtainable under these conditions.

Paper chromatography. Paper partition chromatography was used to check homogeneity of this compound. An apparatus similar to that described by Burton *et al.*¹⁰ was utilized here with the following solvent systems: (1) mobile phase, ethyl acetate-chloroform (1:3) saturated with formamide—immobile phase, formamide-methanol (1:1), Whatman No. 1 paper; (2) 2 per cent K_2HPO_4 with untreated Whatman No. 4 paper; and (3) mobile phase, chloroform saturated with ethylene glycol—immobile phase, ethylene glycol-methanol (1:1), Whatman No. 4 paper. The 2 per cent K_2HPO_4 system used ascending solvent flow. Solutions of chlorpropamide were spotted on paper in amounts containing 50 to 100 γ of compound; at times as much as 1000 γ was streaked on the paper. After development and drying, spots

were detected either by their ultraviolet absorption through a zinc silicate-coated fluorescent screen¹¹ or by spraying with blue tetrazolium reagent.¹² After spraying with the blue tetrazolium reagent, the sheet is warmed a short time in an oven (90° C.); chlorpropamide exhibits a whitish zone on a general blue background. Single zones were observed in these systems. In the

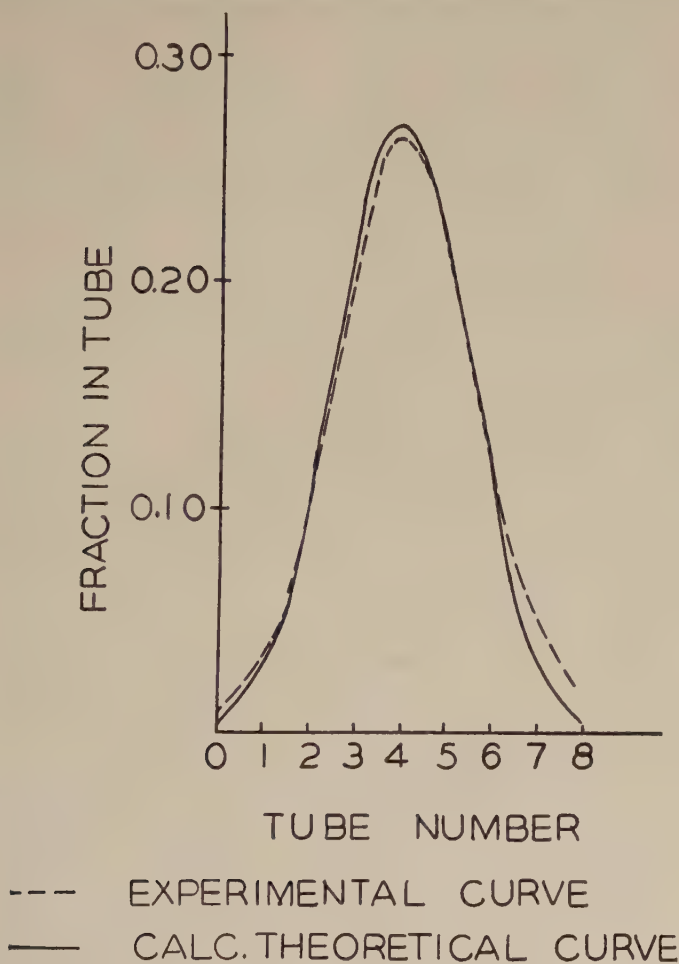


FIGURE 4. Countercurrent distribution of chlorpropamide between chloroform and pH 6.7 buffer.

ethyl acetate-chloroform (1:3) system the R_f value was approximately 0.1; in the 2 per cent K_2HPO_4 system, approximately 0.8-0.9; and in the chloroform-ethylene glycol system, approximately 0.2.

Countercurrent distribution. The homogeneity of chlorpropamide was tested by means of the technique of countercurrent distribution. This compound was distributed between chloroform and Clark and Lubs pH 6.7

buffer. Under these conditions the distribution coefficient of chlorpropamide is about 1. An 8-plate transfer was run, using a chloroform solution of chlorpropamide (concentration of 300 γ /ml.). At the end of the distribution the chlorpropamide content of each phase in each tube was determined through ultraviolet spectrophotometry. When the fraction of the total chlorpropamide content per tube was plotted against the corresponding tube number, a typical distribution curve was obtained in good agreement with a calculated curve (FIGURE 4).

Microanalytical data. Chlorpropamide can be recrystallized readily from acetone-water mixtures. Typical microanalytical data are: calculated for $C_{10}H_{13}N_2O_3ClS$: C, 43.40; H, 4.74; N, 10.12; S, 11.59; Cl, 12.81. Found: C, 43.48, 43.45; H, 4.75, 4.84; N, 10.17, 10.02; S, 11.99, 11.84; Cl, 12.6.

Determination of Chlorpropamide in Human Serum

Reagents. Five reagents were used in this study:

(1) 0.067 M Phosphoric acid: 4.55 ml. of concentrated phosphoric acid (Mallinckrodt, A. R., 85 per cent) diluted to a total volume of 1000 ml. with water.

(2) Chloroform: Mallinckrodt, A. R.

(3) One per cent sodium carbonate: Mallinckrodt, A. R.; 10 gm. of anhydrous sodium carbonate per 1000 ml. of solution.

(4) Hydrochloric acid: (a) 0.4 N, (b) 0.01 N.

(5) Chlorpropamide stock solution: 200 mg. of chlorpropamide are slurried with 30 ml. of distilled water and sufficient 0.1 N NaOH is added (with stirring) until a pH of 7 is reached. The resulting solution is transferred quantitatively to a 100-ml. volumetric flask and diluted to the mark with distilled water. It is stable for at least one week when stored at room temperature. This stock solution serves as a convenient starting point in the preparation of solutions for use in recovery experiments and the determination of the $E_{1\text{cm}}^{1\%}$ value of chlorpropamide.

Apparatus. In this experiment the following apparatuses were used: (1) centrifuge tubes of 10- and 40-ml. capacity, glass-stoppered and graduated; (2) a centrifuge capable of handling the centrifuge tubes; and (3) a spectrophotometer, either the Beckman Model DU spectrophotometer or the Cary Model 11 recording spectrophotometer; quartz cells (1 or 2 cm.) were used for absorbance measurements.

It should be mentioned that clean glassware is absolutely essential throughout this assay procedure. All centrifuge tubes are rinsed with chloroform and dried prior to use.

Procedure. One ml. of serum is mixed with 2 ml. of 0.067 M phosphoric acid and 4 ml. of water in a 40-ml. centrifuge tube. The resulting acidified serum is then extracted with 10 ml. of chloroform by gentle shaking for about 15 seconds.* The phases are separated by centrifugation, and 5 ml. of the

* Occasionally an emulsion forms at this stage which is not easily broken by centrifugation. If this occurs, an additional 10 ml. of chloroform are added to the centrifuge tube and the contents are reshaken and centrifuged. Instead of a 5-ml. aliquot, a 10-ml. aliquot of the clear chloroform layer is taken and the experiment continued as above.

chloroform layer is transferred to a 10-ml. centrifuge tube containing 5 ml. of 1 per cent sodium carbonate. The contents of this centrifuge tube are shaken and then centrifuged. A 4-ml. aliquot of the sodium carbonate layer is transferred to a 10-ml. volumetric flask, neutralized with 2 ml. of 0.4 N hydrochloric acid, and diluted to the mark with 0.01 N HCl. The absorbance value of this solution is determined at 232.5 $m\mu$, using either the Beckman DU spectrophotometer or the Cary spectrophotometer. The reference solvent is 0.01 N hydrochloric acid. As a blank a serum sample (devoid of chlorpropamide) is processed through this same procedure and measured in the same fashion. If a Cary recording spectrophotometer is available, it is well occasionally to record the ultraviolet spectra of the sample and blank over the 220- $m\mu$ to 300- $m\mu$ wave-length interval.

Calculation. Chlorpropamide concentration

$$(\gamma/\text{ml.}) = \frac{(A_S - A_B)_{232.5\text{ }m\mu}(10^6)}{(l)(E_{1\text{ cm.}}^{1\%})(4)}$$

where l is the cell path in centimeters; A_S and A_B are the absorbance values of serum sample and blank; and $E_{1\text{ cm.}}^{1\%}$ is the absorbance of a solution of chlorpropamide in 0.01 N hydrochloric acid at a concentration of 1 gm. per 100 ml., when measured in a 1-cm. cell at 232.5 $m\mu$.

Recovery experiment. Known amounts of chlorpropamide (50 to 250 $\gamma/\text{ml.}$) were added to human serum and then were processed through the above assay procedure. The results of a typical recovery experiment are shown in TABLE 1. Here, recovery is essentially quantitative. Experiments on a

TABLE 1
RECOVERY OF CHLORPROPAMIDE ADDED TO HUMAN SERUM

Chlorpropamide added ($\gamma/\text{ml.}$)	Chlorpropamide found ($\gamma/\text{ml.}$)	Recovery (percentages)
250	248	99
250	248	99
250	248	99
125	127	102
125	123	98
125	123	98
50	51	102
50	50	100
50	51	102

variety of serum samples, to which chlorpropamide had been added in varying amounts (37 to 564 $\gamma/\text{ml.}$), yielded a recovery of 99 ± 1.4 per cent.* It is recommended that recovery experiments should be run so as to thoroughly familiarize one with the assay procedure. In addition, it would be advisable to determine the $E_{1\text{ cm.}}^{1\%}$ value for chlorpropamide under the prevailing laboratory conditions.

* Average \pm average deviation.

Serum blanks. A serum sample obtained from the same subject before the administration of chlorpropamide, when processed through the normal procedure, serves as a convenient serum blank. A variety of serum samples (free of chlorpropamide) from both diabetic and nondiabetic subjects was carried through the above extraction procedure and gave apparent chlorpropamide concentrations of $13 \pm 2.7 \gamma/\text{ml.}$

Reproducibility experiment. Serum samples from several patients being treated with chlorpropamide were combined. Ten individual determinations were made on this pooled serum sample. These results are summarized in TABLE 2.

TABLE 2
REPRODUCIBILITY OF ASSAY ON COMBINED SERUM SAMPLES

Assay No.	Chlorpropamide content ($\gamma/\text{ml.}$)
1	191
2	194
3	191
4	193
5	194
6	191
7	198
8	191
9	195
10	194
	193 ± 1.8

Identification of Chlorpropamide in Human Serum

In order to demonstrate the presence of chlorpropamide in human serum, the following isolation experiment was conducted. Serum samples from 16 diabetic subjects were pooled to provide 30 ml. of serum with a chlorpropamide content of $138 \gamma/\text{ml.}$ This pooled serum sample was acidified to pH 2 to 3 with 0.067 M phosphoric acid and was extracted with chloroform. The resulting chloroform extracts were shaken with an equal volume of 1 per cent sodium carbonate. This 1 per cent sodium carbonate phase was acidified to pH 2 and was extracted with chloroform. The chloroform layer was now shaken with a volume of 1 per cent sodium carbonate such that a fourfold increase in concentration was effected. This 1 per cent sodium carbonate layer was removed, neutralized with 0.4 N HCl, and diluted so as to provide two solutions comparable in concentrations with those shown in FIGURE 2. The ultraviolet absorption spectra of these 2 solutions were recorded on a Cary Model 11 spectrophotometer and were found to be identical with those of chlorpropamide. After the determination of the ultraviolet absorption spectrum, these solutions were combined and extracted with chloroform. The chloroform extracts were concentrated to dryness in a stream of nitrogen and the residue was dried at reduced pressure over potassium hydroxide.

The infrared spectrum of this residue was determined in chloroform in a microcell on a Baird recording spectrometer and it was identical with that of chlorpropamide. Furthermore, the paper chromatographic behavior of this residue was the same as that of chlorpropamide in the 2 per cent K_2HPO_4 system.

Interferences. The method described for the determination of chlorpropamide is not specific for this compound. In order to test for possible interferences, the following compounds were added to 1 ml. of serum in the quantities indicated and were carried through the usual extraction procedure: (1) *p*-chlorobenzenesulfonic acid sodium salt (0.25 mg.); (2) *p*-chlorobenzenesulfonamide (0.25 mg.); (3) acetone (4 mg.); (4) ethyl acetoacetate (5 mg.); (5) β -hydroxybutyric acid (5.5 mg.); (6) acetylsalicylic acid (0.5 mg.); and (7) salicylic acid (0.5 mg.). The ultraviolet absorption spectra of the resulting solutions were examined in the wave-length interval 220 to 350 $m\mu$, using the Cary Model 11 recording spectrophotometer. In the case of sodium *p*-chlorobenzenesulfonate, acetone, and ethyl acetoacetate, the resulting spectra were comparable to that of a serum blank. However, the ultraviolet absorption spectrum of the solution obtained from the β -hydroxybutyric acid experiment exhibited strong end absorption, thus making it difficult accurately to determine chlorpropamide in the presence of 5.5 mg./ml. of this compound. With *p*-chlorobenzenesulfonamide, acetylsalicylic acid, and salicylic acid, major absorption maxima were found at 228 $m\mu$, 227 $m\mu$, and 235.5 $m\mu$, respectively, indicating that these compounds would present a serious interference in the procedure used to determine chlorpropamide.

SUMMARY

Chlorpropamide is an acidic substance as demonstrated by potentiometric titration. Its ultraviolet absorption spectrum shows a maximum at 232.5 $m\mu$ in 0.01 N hydrochloric acid, with less intense maxima in the 250 to 280 $m\mu$ wave-length region. The infrared absorption spectrum of chlorpropamide is characterized by a number of strong absorption bands through the 2 to 15 μ wave-length region. It is soluble in a variety of common organic solvents, but exhibits a limited solubility in water at *pH* values below 5. Its homogeneity can be demonstrated by paper partition chromatography and counter-current distribution.

A method is presented for the determination of chlorpropamide in human serum. It consists of a two-stage extraction procedure that includes an extraction of acidified serum with chloroform, followed by re-extraction of the chloroform phase with dilute sodium carbonate. After suitable acidification of the sodium carbonate phase, the chlorpropamide content is determined by measurement of the absorbance at 232.5 $m\mu$. Recoveries of chlorpropamide from human serum and serum blank values are quite acceptable. The manipulations involved in the assay are relatively simple, and determinations can be carried out in reasonably short time intervals. Chlorpropamide was isolated from serum by a multiple extraction procedure and then identified via its ultraviolet and infrared absorption spectra and paper chromatography. The method of assay lacks specificity; compounds such as

β -hydroxybutyric acid, *p*-chlorobenzenesulfonamide, acetylsalicylic acid, and salicylic acid would present interferences.

ACKNOWLEDGMENTS

We acknowledge our sincere appreciation to T. M. Lees, G. W. Walsh, F. R. LaPietra, R. J. Hickey, W. H. Boegemann, and H. J. Pazdera of the Chas. Pfizer & Co., Inc., staff for their contributions to the work described in this paper.

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METABOLIC FATE OF CHLORPROPAMIDE IN MAN*

Philip C. Johnson, Allen R. Hennes, Theda Driscoll, Kelly M. West
*Radioisotope Service and Medical Service, Veterans Administration Hospital, and
Department of Medicine, University of Oklahoma, Oklahoma City, Okla.*

We have investigated the urinary excretion, blood levels, protein binding, and chromatographic behavior of chlorpropamide, a new sulfonylurea. This was accomplished by administering this drug to nondiabetics and to diabetic patients. Work by others has shown that tolbutamide, another sulfonylurea, has a serum half time of 4 hours, and that it is carboxylated at the *p*-methyl group *in vivo*.^{1,2} On the other hand, carbutamide has a longer serum half time of 33 hours, and the *p*-amino group is acetylated *in vivo*.¹ To our knowledge there are no published reports concerning the metabolic fate of chlorpropamide in man.

Methods

The radioactive chlorpropamide used in these experiments had an initial specific activity of 66.6 mg./mc. It was given as a water solution with sufficient carrier added so that 20 μ c of sulfur-35 equaled 250 mg. of chlorpropamide. This solution was given to patients as follows.

Group 1. Four nondiabetic patients without renal disease were given a single oral dose of 250 mg. of radioactive chlorpropamide containing 20 μ c of sulfur-35.

Group 2. Three stable diabetic patients: subject No. 1, 45 years old, received 500 mg. tolbutamide twice daily; subjects Nos. 2 and 3, 71 and 64 years old, respectively, received 250 mg. of chlorpropamide daily as therapy. On the day of the test each patient received orally one dose of 250 mg. of radioactive chlorpropamide. No further chlorpropamide was given during the test period.

Group 3. (a) A 41-year-old diabetic patient who had not received chlorpropamide prior to this test was given intravenously 20 μ c of radioactive chlorpropamide without added carrier. One tablet of 250 mg. of chlorpropamide was given orally at the same time. (b) A 71-year-old diabetic was treated in the same way as the patients of Group 1.

The radioactive samples were counted with an automatic micromil gas flow G.M. tube with an average background of 14 cpm, using a preset count of 1000. Sufficient recounts were obtained to give a 5 per cent average counting error or, in the case of the weaker samples, a sufficient number of recounts to obtain at least 3 count rates that were within 1 cpm of the median count rate. The mean of these median values was considered to be the true count rate. Each sample was weighed and corrected for self absorption. All serum samples were deproteinized by sodium tungstate and sulfuric acid precipitation prior to plating.

* The work reported in this paper was supported in part by a grant from Chas. Pfizer & Co., Inc., Brooklyn, N. Y.

The binding of chlorpropamide was determined *in vitro* by adding 25 to 200 μg . of chlorpropamide containing 0.01 μc of sulfur-35 to 1-ml. aliquots of serum. These aliquots were diluted to 4 ml. with saline and incubated at 37° C. for 24 hours. The incubated aliquots were dialyzed in cellulose tubing at pH 7.6 for 48 hours. The per cent of the radioactivity remaining at the end of the dialysis was considered as the percentage of bound chlorpropamide. The binding of 4 types of protein solutions was determined as follows: (1) normal serum; (2) commercial serum albumin in 2 concentrations, 25 and 50 mg./ml.; and (3) serum obtained from a patient with marked hypoalbuminemia. His serum protein was 4.7 gm. per cent. Electrophoretic analysis showed that 2.6 per cent of this protein was albumin, 6.5 per cent alpha-1-globulin, 17 per cent alpha-2-globulin, 23 per cent beta globulin, and 51 per cent gamma globulin. *In vivo* binding was determined by giving a diabetic patient 250 mg. of radioactive chlorpropamide orally. Serum samples were obtained at 4 and 48 hours. These samples were dialyzed in the same way as the protein solutions.

The chromatographic method used for the detection of chlorpropamide in blood and urine was a modification of the method of Seligson and Shapiro for α -keto acids.³ The only significant change was elimination of the incubation with 2-4 dinitrophenylhydrazine. A chlorpropamide standard was used to locate the chlorpropamide spots under ultraviolet light after chromatography. Numerous unidentified spots seen under ultraviolet light after chromatography of extracts of urine are unrelated to the administration of chlorpropamide.

To determine whether erythrocytes absorbed chlorpropamide, we incubated 25-ml. samples of fresh whole blood at 37° C. for 2 hours in siliconized vessels gassed with 5 per cent CO₂ and 95 per cent O₂. The plasma was separated by centrifugation. The erythrocytes were resuspended and washed in cold normal saline 4 times prior to the determination of radioactivity.

The serum concentration of chlorpropamide was measured by the technique of Toolan and Wagner.⁴ This method makes use of a chloroform extraction of acidified serum with 0.067 M phosphoric acid. The chloroform extract is then washed with aqueous 1 per cent sodium carbonate. The aqueous sodium carbonate is acidified to pH 2 with HCl, and the optical density is measured at 232.5 $m\mu$. The optical density of the fasting serum is subtracted and the net optical density is compared to a standard. The average optical density of the serum of 5 normals who had not received this drug previously gave an apparent chlorpropamide value of 17 μg ./ml. by this method.

Results

TABLE 1 contains the serum radioactivity values obtained from 4 non-diabetic patients following the ingestion of 250 mg. of chlorpropamide containing radioactive sulfur-35. Maximum serum radioactivity and optical density were reached during the first 2 hours in each case. The log of the radioactivity is plotted against time in FIGURE 1; a line connects the mean values. It is obvious that 2 mathematical functions exist. The first and

more rapid of these has an uncorrected half time of 32 hours; the slower component has a half time of approximately 16 days. However, since only 2 data points were obtained, this value is only a rough estimate. If the effects of the slower component are removed from the initial slope, a half time of approximately 24 hours is obtained. These data show that the serum

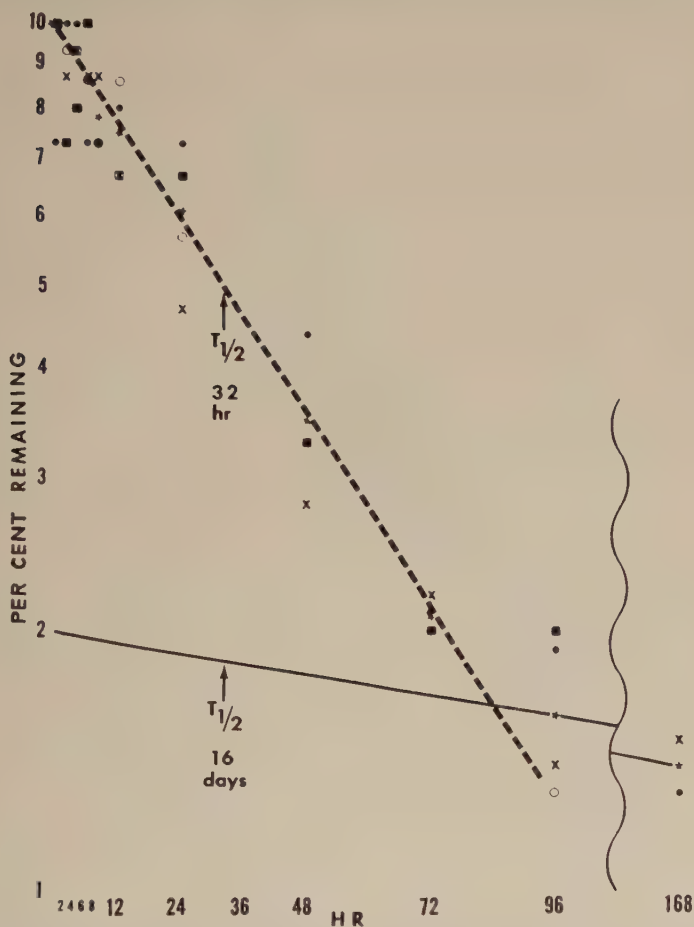


FIGURE 1. Disappearance of serum radioactivity from the serum of 4 nondiabetic patients. In this figure, 10 is equal to 100 per cent.

disappearance of chlorpropamide occurs at 2 separate rates. Half times determined by measuring the disappearance of chlorpropamide from the serum, using the chloroform soluble optical density, were 20 hours for subject No. 1, and 24 hours for subject No. 3. This confirmed the disappearance half times obtained using the radioactive data.

TABLE 2 shows the results obtained from 5 diabetics. The first 3 subjects had all been on the drug up to the time of the start of this test. The fourth

TABLE 1
SERUM RADIOACTIVITY CORRECTED TO PEAK ACTIVITY

Disappearance of radioactive chlorpropamide from the serum of 4 nondiabetic patients. The maximum serum radioactivity has been made equal to 100. The actual maximum serum radioactivity is listed at the bottom of the table

Hours	Subject No.				Mean
	1	2	3	4	
1	100	100	73	100	
2	87	93	100	73	
4	93	93	100	80	
6	87	86	73	100	86
8	87	73	73		78
12	67	86	80	67	75
24	47	57	73	67	61
48	28	36	44	33	35
72	21	21	21	20	21
96	14	13	19	20	16
168	15		13		14
Net cpm/ml. serum at 100%...	90	84	90	81	

TABLE 2
SERUM RADIOACTIVITY CORRECTED TO PEAK ACTIVITY

Disappearance of radioactive chlorpropamide from the serum of diabetic patients. The maximum serum radioactivity was made equal to 100. The actual maximum serum radioactivity is listed at the bottom of the table

Subject No.....	Group No. 2			Group No. 3	
	1	2	3	1	2
Hours					
1	100	55	92	100	82
2	85	100	100	76	100
3				92	
4	85	64	83		88
5				76	
6	100	64	75		82
8	85	73	83	72	70
24	77	54	50	64	59
48	69	45	42	40	41
72	46	36	25	28	35
96	54	36	25	20	35
Net cpm/ml. serum equal to 100%.....	60	66	72	150	72

subject received this drug intravenously, and the fifth subject received this drug in the same way as the patients of Group 1; the data from this table are shown in FIGURE 2. It can be seen that the early disappearance of the radioactivity from the serum in all but one subject was similar to that of the nondiabetic patients given this drug. However, all values fall outside of 2

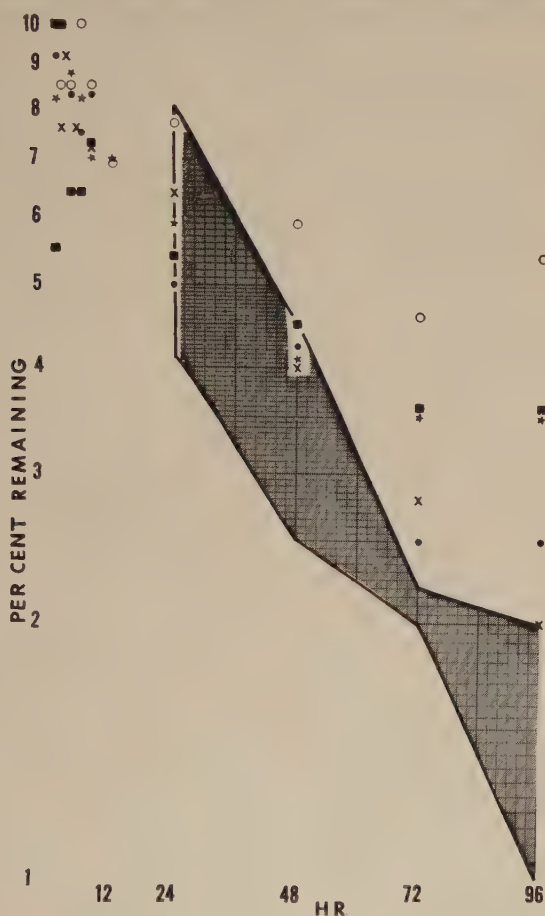


FIGURE 2. Disappearance from the serum of radioactive chlorpropamide in 5 diabetic subjects. In this figure, 10 is equal to 100 per cent.

standard deviations from the mean of the normal group after the 48-hour specimen. Whether this higher percentage of slowly excreted chlorpropamide represents differences between the handling of the drug in normals and diabetics cannot be determined from these data since the groups are not comparable. It is notable that the disappearance of the radioactivity from the serum of subject No. 1 was much slower than that of the other subjects. This diabetic patient, preloaded with tolbutamide, had a normal phenolsulfonphthalein excretion, urinalysis, and blood urea nitrogen. His serum

uric acid level was elevated, but there was no other evidence of gout except that he had had an episode of pain in the right ankle of several days' duration on one occasion 2 months prior to these studies.

TABLE 3 presents the percentage of the ingested dose excreted in the urine of the nondiabetic subjects in Group 1. The patients are presented in the same order as they were in TABLE 1 so that a comparison can be made between

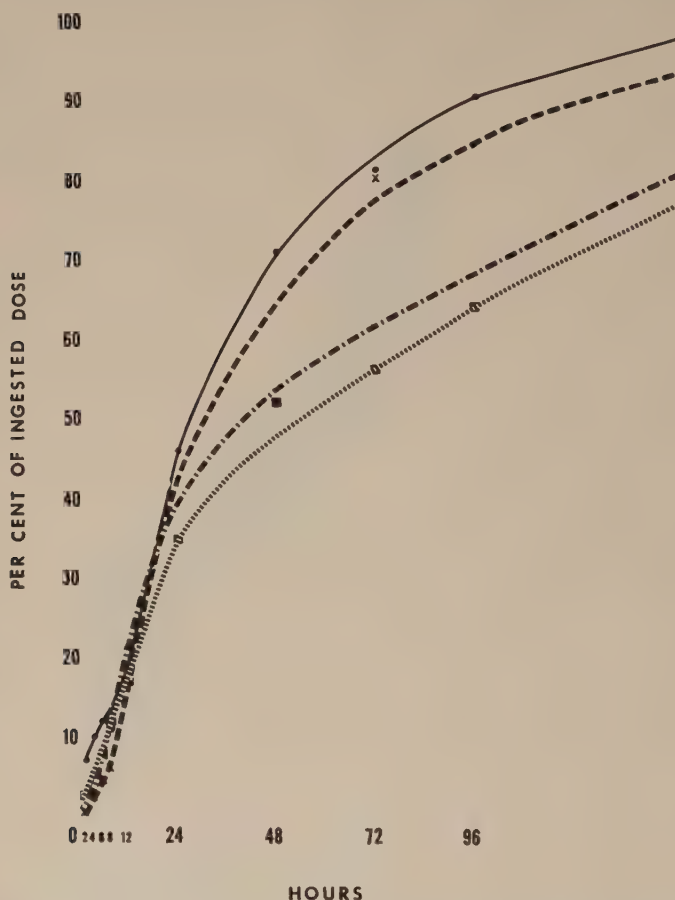


FIGURE 3. Cumulative urinary excretion of radioactive chlorpropamide in 4 non-diabetic patients.

the serum and urinary radioactivity. FIGURE 3 shows these data plotted linearly. The mean cumulative excretion during the 96 hours of the tests was 77 per cent. This agrees well with the serum values, which showed 16 per cent of the radioactivity remaining in the serum 96 hours after the ingestion of the chlorpropamide. This suggests that in the patients without diabetes almost all of the excretion is accounted for in the urine.

TABLE 4 and FIGURES 4 and 5 show the urinary excretion of the radioactivity

TABLE 3

CUMULATIVE URINARY EXCRETION OF RADIOACTIVE CHLORPROPAMIDE BY FOUR NONDIABETIC SUBJECTS

The values presented are the percentages of the ingested dose

Subject No...	1	2	3	4
Hours				
2	0.7	1.3	7.1	2.6
4	2.7	3.6	10	5.2
6	4.9	8.4	12	7.8
8	6.4	11	13	12
12	17	21	19	17
24	42	41	46	35
48	60	54	71	52
72	80	62	81	56
96	84	68	90	64

TABLE 4

CUMULATIVE URINARY EXCRETION OF RADIOACTIVE CHLORPROPAMIDE BY FIVE DIABETIC SUBJECTS

The results are presented as percentages of the ingested dose

Subject No.....	Group No. 2			Group No. 3	
	1	2	3	1	2
Hours					
2	0.4	-	1.2	3.0	3.9
4	1.7	0.5	3.4	8.2	5.1
6	3.0	1.3	4.7		13
8	3.7		6.8	12	15
12	4.5	3.0	11	17	22
24	8.5	7.8	19	36	35
48	17	18	36	66	55
72	24	27	43	88	66
96	31	34	47	92	75
120		47	61		79

obtained from the diabetic patients of Groups 2 and 3. Their serum values are presented in the same order as in TABLE 2. In FIGURE 4 the urinary excretion of these preloaded patients was markedly less than that of the normal group. However, it should be noted that the 2 groups are not completely comparable in regard to certain other factors such as age and the presence of diabetes. Consequently, the differences in the preloaded group and the group not loaded prior to testing could be due to other factors. The mean cumulative urinary excretion of the nondiabetics of Group 1, plus and minus 2 standard deviations, is presented on this figure for comparison.

FIGURE 5 shows the cumulative urinary excretion of the other 2 diabetic

patients of Group 3. The patient with the highest urinary excretion received this drug as therapy up to the day of the test. On the day of the test he received an intravenous tracer dose of chlorpropamide without added carrier, followed by a 0.25-gm. tablet of chlorpropamide. His rapid excretion of the drug is markedly different from that of the other 3 diabetics. The other excretory curve was obtained from a patient given 1 oral dose of this drug.

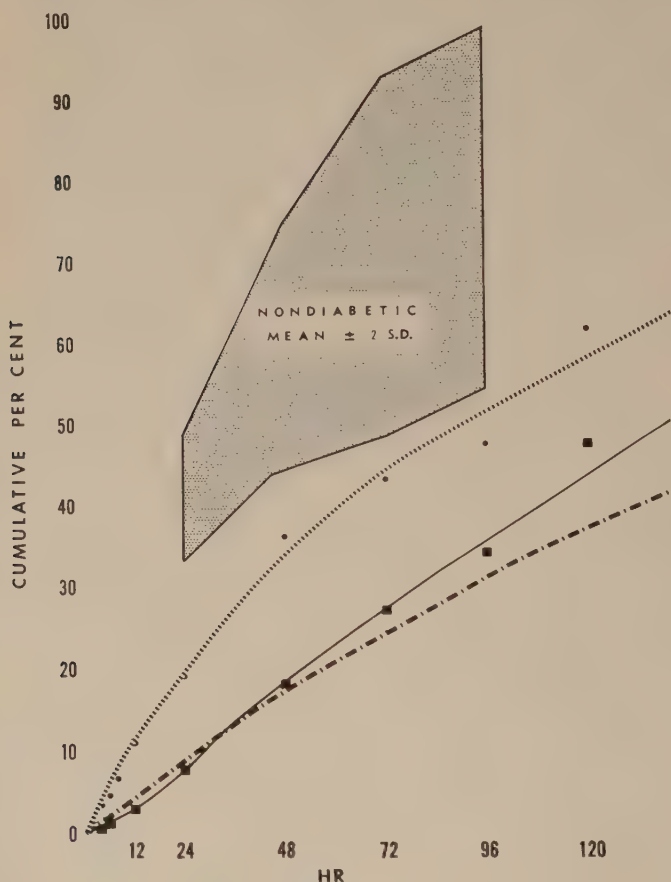


FIGURE 4. Cumulative chlorpropamide urinary excretion of 3 diabetic subjects pre-loaded with chlorpropamide or tolbutamide. The mean \pm 2 S.D. of the mean of the normal group is presented for comparison.

His excretory pattern is very similar to that obtained from the 4 patients in the normal group.

Fecal excretion of an intravenously injected chlorpropamide was determined in 1 diabetic patient who was already taking this drug as therapy. Carmine red dye given at the same time as the tracer dose appeared in his 48-hour stool specimen. The patient's fecal excretion of chlorpropamide was 0.52 per cent on day 1, 0.92 per cent on day 2, and 0.74 per cent on day 3.

It would appear that fecal excretion is not a very significant excretory pathway.

TABLE 5 shows the results of our dialysis experiments. *In vitro* binding by a normal subject was 6.9 per cent. A patient with a total serum protein of 4.7 gm. per cent with almost no albumin showed binding of 3.1 per cent, which is less than one half of that of the normal serum. Albumin solutions

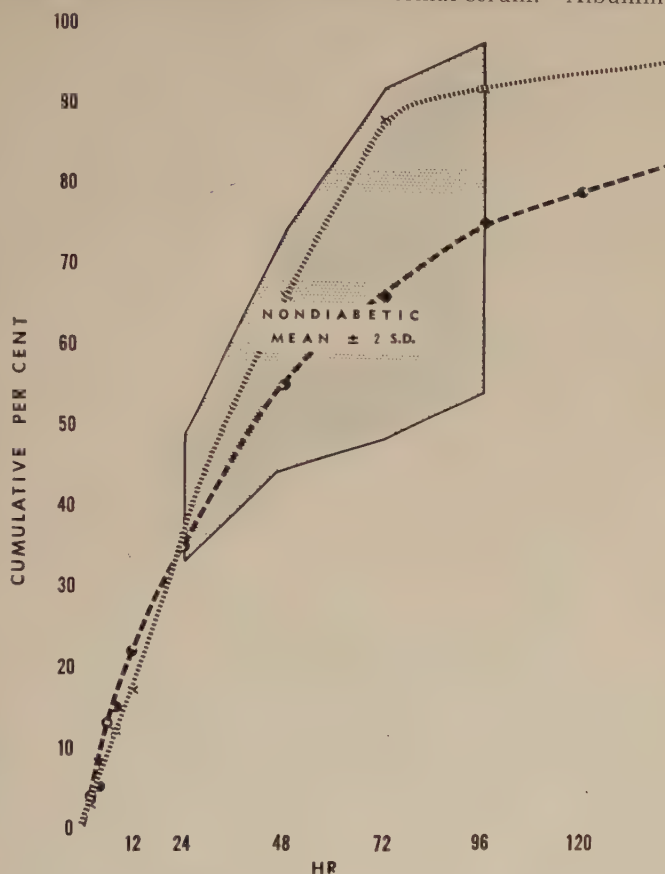


FIGURE 5. Cumulative chlorpropamide urinary excretion of 2 diabetic subjects. The mean ± 2 S.D. of the mean of the normal group is presented for comparison.

of 2.5 and 5.0 gm. per cent bound 2.2 and 4.6 per cent, respectively. These data suggest that both albumin and globulin participate in this binding. However, no complete serum fractionation studies have been done. We did not find binding of chlorpropamide by crystalline insulin solutions of 1 to 4 mg./ml. (22 units/mg.). These concentrations of insulin are considerably less than those of the other protein solutions; therefore binding of a degree equal to that of albumin could have been missed. Nondialyzable chlorpropamide was present in the serum of a diabetic patient who had ingested this drug. These values approximate those of the *in vitro* experiments.

TABLE 5
PROTEIN BINDING OF RADIOACTIVE CHLORPROPAMIDE MEASURED BY DIALYSIS
Determined by dialyzing serum protein samples at pH 7.6 for 48 hours

	Per cent bound
	(Mean \pm standard error)
<i>In vitro</i>	
Normal.....	6.87 \pm 0.66
Hypoalbuminemic.....	3.08 \pm 0.15
Albumin 2.5 gm. %.....	2.23 \pm 0.08
Albumin 5.0 gm. %.....	4.60 \pm 0.33
<i>In vivo</i>	
Diabetic 4 hours.....	4.35 \pm 0.60
Diabetic 48 hours.....	6.96 \pm 0.99

TABLE 6
EFFECT OF INCREASING CONCENTRATIONS OF CHLORPROPAMIDE ON PROTEIN BINDING
Each sample was incubated for 24 hours at 37° C. and dialyzed at pH 7.6 for 48 hours

Concentration (μ g./ml.)	Normal serum		Serum albumin (50 mg./ml.)	
	Per cent	μ g.	Per cent	μ g.
25	7.8	1.9	6.1	1.5
50	6.6	3.3	6.6	3.3
100	4.9	4.9	4.9	4.9
200	13	26	11	22

In an attempt to determine the effect of varying concentrations of chlorpropamide on these binding phenomena, we added 25 to 200 μ g. of chlorpropamide to 2 protein solutions. TABLE 6 shows that varying the concentration of chlorpropamide resulted in a nearly constant percentage of chlorpropamide remaining after dialysis. However, the micrograms bound increased with increasing amounts of chlorpropamide.

With the chromatography system used by us the sulfur-35-labeled chlorpropamide standard exhibited 3 separate radioactive areas. Little difference was noted in the R_f values of the standard and that obtained from serum and urine specimens. The R_f values obtained are shown in TABLE 7. Seventy to 85 per cent of the radioactivity was associated with the R_f value 0.9 in the standard and in the extracts of urine and serum. This area of radioactivity corresponds to the major area of ultraviolet light absorption. We have also failed to produce a precipitate in urine specimens containing excreted chlorpropamide by reducing the pH to 1. It would appear that little if any structural modification of chlorpropamide occurs *in vivo*.

The incubation of chlorpropamide with blood resulted in a plasma radio-

TABLE 7
CHROMATOGRAPHY OF SULFUR-35-LABELED CHLORPROPAMIDE

Chromatography of serum and urine specimens following the ingestion of chlorpropamide. Peak No. 1 contained 70 to 85 per cent of the radioactivity, Peak No. 2 contained 10 to 25 per cent of the radioactivity, and Peak No. 3 contained 0 to 5 per cent of the radioactivity

Peak No.....	1	2	3
Chlorpropamide standard.....	0.9	0.4	0.2
2-Hour serum.....	0.8	0.5	
8-Hour serum.....	0.9	0.3	
2-Hour urine.....	0.9	0.4	0.2
8-Hour urine.....	0.9	0.4	

activity of 15,730 net counts per minute (ncpm) and an erythrocyte radioactivity of 70 ncpm. After 2 hours of incubation the plasma count was 16,120 ncpm, and the erythrocyte count was 100 ncpm. This slight difference in radioactivity in the erythrocytes could be accounted for easily by experimental variations in the washing of the erythrocytes. In confirmation of these data, erythrocytes obtained from a patient given an oral dose of radioactive chlorpropamide failed to show absorbed radioactivity.

Discussion

The disappearance of radioactive chlorpropamide from the sera of the 4 nondiabetic patients in Group 1 showed at least 2 separate disappearance rates. These dual disappearance phenomena exhibited by chlorpropamide could be explained either by serum protein binding or by chemical alteration. The apparent initial disappearance rate was 32 hours, which is similar to that of carbutamide. The second and slower disappearance rate of 16 days has not been reported to occur following the ingestion of carbutamide and tolbutamide.

Extrapolation of the slower component back to zero time suggests that approximately 20 per cent of chlorpropamide could be present as this slowly excreted component; this is 3 times greater than values obtained in the binding experiments. Whether this slower component merely represents chlorpropamide bound to serum proteins or metabolized chlorpropamide cannot be determined from these data. Our evidence would suggest that this slowly excreted component plays a greater role in the diabetic patients who have been receiving chlorpropamide as therapy. Perhaps chronic administration causes a difference in the rate of excretion or an increase in binding. The markedly prolonged excretion of chlorpropamide by diabetic patient No. 1 shows that significant differences in the time required to excrete this drug exist among diabetic patients receiving this drug as therapy.

The urinary excretion of radioactive chlorpropamide closely parallels its disappearance from the serum. The excretion of chlorpropamide by the preloaded diabetic patients was not as uniform as that of the nondiabetic patients. This would suggest that significant differences exist in the excre-

tion of this drug by various diabetic patients. Because of the possible clinical importance of these differences and because this may be related to the diabetic state, further study should be made of this phenomenon. Unlike carbutamide or tolbutamide, this compound does not seem to be metabolized prior to excretion, since no definite chromatographic differences were noted among serum, urine, and standard.

Binding of carbutamide and tolbutamide by serum proteins has not been reported. Chlorpropamide appears to be bound to both serum albumin and globulin. This binding is related to the concentration of chlorpropamide present in the serum, and approximately equal binding can be produced *in vitro*. The presence of binding by proteins may account for the odd dilution-space determinations obtainable after ingestion of this drug. By determining the space of distribution of intravenously administered chlorpropamide in two 70-kg. diabetic patients, 10 and 11 l. spaces were obtained. These values do not correspond to either the extracellular or intravascular space, and correcting it for amount of chlorpropamide bound by the serum proteins still fails to produce a space of distribution comparable to those reported for carbutamide or tolbutamide. We can surmise that binding to proteins other than serum proteins or leakage into the cells must occur. Our failure to find absorption of chlorpropamide by *in vitro* incubation of erythrocytes does not rule out concentration of this drug by other tissues.

Summary

(1) We have used chlorpropamide labeled with sulfur-35 to determine the metabolic fate of this drug.

(2) The disappearance of chlorpropamide has an initial apparent half time of 32 hours for the major portion of the dose and a half time of 16 days for the remainder. Chlorpropamide is excreted almost entirely by the kidneys. Only very slight fecal excretion was demonstrated.

(3) Chlorpropamide is made partially nondialyzable by serum proteins. Increasing the concentrations of chlorpropamide in the serum increases this binding.

(4) We could not detect metabolic alteration of chlorpropamide prior to excretion.

Acknowledgments

The technical assistance of Thomas Redding, Ronald Lee, and Beulah Wulff, and the art work of Shirley Shipman are greatly appreciated.

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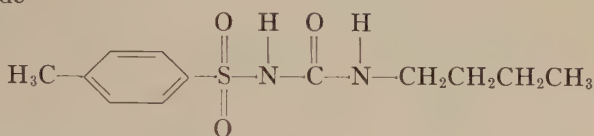
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Discussion of the Paper

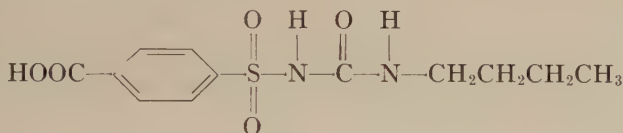
STEFAN S. FAJANS (*University of Michigan, Ann Arbor, Mich.*): In view of the almost complete excretion of chlorpropamide in urine reported by Philip Johnson, I shall mention briefly some published and unpublished observations about the metabolism and excretion of tolbutamide.

In 1956 we reported upon the findings of a major urinary excretion product of tolbutamide after its administration to normal and diabetic subjects. Upon acidification of the urine of such subjects to a *pH* 5 or less, a white precipitate appeared. This was shown to be the carboxylic acid derivative of tolbutamide (1-butyl-3-*p*-carboxyphenyl-sulfonylurea), as reported by us in conjunction with Struck and others of the Upjohn Research Laboratories, Kalamazoo, Mich.

Tolbutamide



Tolbutamide excretion product



On very large dosages of tolbutamide, such as 6 gm. per day, a maximum of about 75 per cent of the administered drug could be recovered from the urine as this metabolic oxidation product. This was determined by acidifying the urine and then drying and weighing the purified insoluble precipitate.

In order to rule out additional urinary metabolites of tolbutamide and to determine more quantitatively the urinary excretion of the carboxyl compound, a joint project was set up with W. L. Miller of the Upjohn laboratories at his suggestion. It was planned to give S^{35} -tolbutamide to normal and diabetic subjects and to examine the urine for metabolites of tolbutamide by paper chromatography.

As can be seen from TABLE 1, 3 gm. of unlabeled tolbutamide was given daily to 6 subjects for 5 days, and 15 mg. of S^{35} -tolbutamide was administered on the fourth day. Urines were collected for 24 hours on the fourth and fifth day. The chromatographic studies were performed by W. L. Miller.

The results of these studies are given in the table. It is apparent from the data for the first 4 subjects (3 control subjects and 1 tolbutamide non-responsive diabetic subject) that 90 per cent or more of the administered drug was excreted as the carboxyl sulfonylurea within the first 24 hours, and nearly 100 per cent within 48 hours. Furthermore, the carboxyl excretion product was the only human transformation product of tolbutamide identified.

The lesser amounts of radioactivity recovered in the urine of the last two diabetic patients could well be accounted for by unfortunate errors in urine collection on the hospital wards. In these two patients there was also no evidence of excretion of a metabolite other than the carboxyl-substituted tolbutamide.

In summary, it can be stated that:

(1) the carboxylic acid derivative of tolbutamide is the only urinary excretion product in man, and (2) on the basis of data from the first four subjects, it appears that tolbutamide is quantitatively excreted in the urine as this metabolite.

TABLE 1
URINARY EXCRETION OF S^{35} -CARBOXYL SULFONYLUREA AFTER ORAL ADMINISTRATION OF S^{35} -TOLBUTAMIDE*

Subjects	S^{35} Excretion, per cent		
	0 to 24 hours	24 to 48 hours	0 to 48 hours
Normal control			
T. D.	99.0	1.0	100.0
G. R.	95.6	4.3	99.9
J. D.	89.8	10.0	99.8
Tolbutamide-nonresponsive diabetic			
R. J.	90.0	9.0	99.0
Tolbutamide-responsive diabetics			
R. C.	—	—	90.6
N. M.	73.1	6.9	80.0

* Tolbutamide was administered 3 gm. per day for 5 days; S^{35} -tolbutamide, 15 mg. on fourth day. Urine collections were made on fourth day (0 to 24 hours) and fifth day (24 to 48 hours).

RELATIVE POTENCIES OF CHLORPROPAMIDE AND TOLBUTAMIDE IN MAN

Kelly M. West and Stanley R. McCampbell

*Department of Medicine, University of Oklahoma School of Medicine and the Veterans
Administration Hospital, Oklahoma City, Okla.*

In a previous publication¹ we have reported the results of experiments in which the hypoglycemic potencies of chlorpropamide and tolbutamide in 10 normal subjects were compared at varying intervals after administration of the drugs by mouth. One gram of chlorpropamide produced responses that exceeded those to 1 gm. of tolbutamide at 1, 2, 3, 4, 6, 12, and 18 hours after administration. More recently the venous blood glucose levels² of 20 normal subjects were determined 1 hour after each subject had received 1 gm. of tolbutamide on one occasion, and after 1 gm. of chlorpropamide on another occasion, at intervals of 1 week. The order in which the drugs were administered was alternated. The drugs were administered orally in tablet form together with 4 ounces of water. The mean difference between the control fasting blood glucose values and the blood glucose levels 1 hour after tolbutamide averaged 6.6 mg. per cent, while chlorpropamide produced responses averaging 14.1 mg. per cent. This difference was highly significant ($p < .001$). Since it seems unlikely that either drug had been appreciably metabolized or excreted as early as 1 hour after administration by mouth, the greater potency of chlorpropamide is probably not due entirely to its slower rate of degradation and excretion. The fact that chlorpropamide was definitely more potent 1 hour after administration suggests the possibility that it is more potent than tolbutamide even when serum levels are comparable. However, the levels of the drugs in the serum were not determined in this experiment. Consequently, it is possible that the greater potency of chlorpropamide is partially due to the fact that it is more readily or more completely absorbed.

FIGURE 1 shows the responses of 6 normal subjects to varying doses of chlorpropamide and tolbutamide when the blood glucose levels were measured 2 hours after the doses were administered in random order at intervals of not less than 4 days (those subjects who received 2 gm. of chlorpropamide were not given another dose for at least 1 week). Each of the subjects received 4 different doses of each drug, with the exception that only 3 of the subjects received a dose of 2 gm. of chlorpropamide. In each instance the fasting blood glucose was compared to the blood glucose 2 hours after a drug.

It may be noted that, with each of the doses, the responses were greater to chlorpropamide than to tolbutamide. Tolbutamide, 0.25 gm., produced no response, while 0.5 gm. of the drug produced responses comparable to those after 0.25 gm. of chlorpropamide. Tolbutamide, 1 gm., and chlorpropamide, 0.5 gm., also produced similar responses. It is of interest that tolbutamide, 2 gm., produced responses that were not greater than those to 1 gm., which suggests the possibility that the point of maximum response was being

reached. However, the data are not adequate to establish this point. On the other hand, the responses to the various doses of chlorpropamide were roughly related to the log of the dose.

Since the biological half time of chlorpropamide is several times longer than that of tolbutamide (Johnson *et al.*, in this monograph), it seems likely that differences in the responses to chlorpropamide and tolbutamide would be far greater than those demonstrated here if the effects were measured 12 to 48 hours after comparable doses of the 2 drugs.

That the high serum levels present for several days after a single dose of chlorpropamide are associated with proportionate physiological activity of

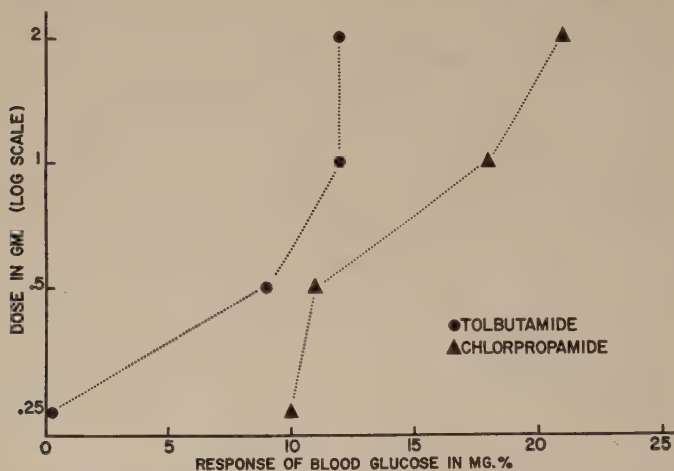


FIGURE 1. Hypoglycemic potencies of tolbutamide and chlorpropamide in normal subjects. Each plot represents the mean response of 6 subjects 2 hours after the oral administration of the drug, except that only 3 of the subjects received 2 gm. of chlorpropamide. The patients who received chlorpropamide also received tolbutamide. See text for details.

the drug is suggested by the following example. We administered chlorpropamide, 2 gm., to one of our normal subjects, whose blood glucose was measured 1 hour later and proved to be 44 mg. per cent. At 2 hours it was 49 mg. per cent and, at the end of the 2-hour experiment, he was given a high-carbohydrate feeding and subsequently took frequent feedings for a period of 64 hours. At 7:00 A.M., 71 hours after administration of the drug, he could not be entirely aroused from sleep. The state of semiconsciousness persisted for 1 hour before being relieved immediately by the intravenous injection of 50 gm. of glucose. The level of serum chlorpropamide at this time was 16.4 mg. per cent.

Serum Levels of Orally Administered Chlorpropamide

TABLE 1 shows the levels of chlorpropamide in serum (Johnson *et al.*, in this monograph) 1 and 2 hours after a 1-gm. tablet of chlorpropamide was administered with 4 ounces of water to each of 7 normal subjects. It is of interest

to note that the very high levels achieved indicate that more than one third of the administered dose was accounted for in the plasma. This suggests that either the drug does not move freely through the capillary walls or that it is in some way partially bound to a substance or substances in the plasma. Elsewhere in this volume, Johnson *et al.* indicate that chlorpropamide is to some extent bound to protein. If this is the case, it is probably not appropriate to refer to a chlorpropamide "space." If, however, it were assumed that the drug was homogeneously distributed in a space, the data in TABLE 1 would indicate that such a space would necessarily be less than 9 l. in volume, since there is at least 115 mg. in every liter of plasma 2 hours after oral administration of 1 gm. of the drug.

The Effect of Chlorpropamide on Glucose Tolerance

One of the strongest arguments against the direct or indirect peripheral action of the sulfonylurea agents has been the failure to demonstrate an effect of single doses of tolbutamide on glucose tolerance.^{4, 5} On the other hand, TABLE 2 shows that chlorpropamide produced in each of 5 subjects a statistically significant increase in the rate of disappearance of intravenously administered glucose. Venous blood glucose determinations were carried out 10 min. and 40 min. after the start of an injection of 15 gm. of glucose injected in 1 to 2 min. The rate at which the glucose disappeared was then calculated and expressed as per cent per minute disappearance.⁵ On a second occasion the test was repeated in the same manner, except that chlorpropamide, 0.5 gm., was injected with the glucose. The control mean disappearance rate was 1.22 per cent per minute. After chlorpropamide, the mean disappearance rate was 1.85 per cent per minute. One of these subjects, weighing 178 pounds, had a control rate of 1.6 per cent per minute, which increased 2.4 per cent per minute after chlorpropamide, 0.5 gm. It is of interest that the

TABLE 1
SERUM LEVELS OF CHLORPROPAMIDE AFTER 1 GM. ORALLY

Subject	Weight (lbs.)	Serum chlorpropamide	
		1 Hour (mg. %)	2 Hours (mg. %)
1	155	3.6	13.0
2	145	5.8	10.9
3	124	2.9	10.6
4	178	6.9	12.0
5	185	8.3	12.7
6	114	7.0	10.3
7	145	8.2	10.7
Mean.....	149	6.1	11.5

intravenous administration to this subject of 1.8 units of glucagon-free insulin produced a strikingly similar change (2.5 per cent per minute).

Discussion

It is not possible to establish accurately the ratio of the potencies of chlorpropamide and tolbutamide from the data in FIGURE 1. In the first place, too few subjects have been tested to establish precisely a dose-response curve for either drug. However, it is clear that in a certain range of dosage chlorpropamide produced an effect approximately comparable to that produced by doses of tolbutamide twice as large. As mentioned previously, it may be assumed that the difference in the responses to chlorpropamide and tolbutamide on a milligram-for-milligram basis could be expected to be much greater if the tests were done from 12 to 48 hours after administration of the drugs because of the longer biological half time of chlorpropamide (Johnson *et al.*, in this monograph). The difference in potency might be expected to be particularly marked if the response to the drugs were compared after daily administration, in which case there would be a cumulative effect for a few days with chlorpropamide and no appreciable cumulative effect with tolbutamide.

It should be stressed that the subjects who received chlorpropamide (FIGURE 1) were the same who received tolbutamide. This paired-control design will, of course, make it possible to identify significant differences in the dose-response curve with a fewer number of tests, since the factor of the varying responsiveness from subject to subject is eliminated. At present we are doing a paired-control study in which responses of normal subject to 0.25 gm. chlorpropamide are being compared to their responses to 0.5, 1, and 2 gm. of tolbutamide. This experiment will continue until statistically significant differences are identified.

The degree to which studies of potencies of sulfonylurea derivatives in normal subjects are applicable to the establishment of their potencies in various

TABLE 2
THE EFFECT OF 0.5 GM. CHLORPROPAMIDE ON INTRAVENOUS GLUCOSE TOLERANCE

Fasting blood glucose (mg. %)		10 min. after glucose* (mg. %)		40 min. after glucose (mg. %)		Glucose disappearance rate, % per minute†	
Control	Chlorprop.	Control	Chlorprop.	Control	Chlorprop.	Control	Chlorprop.
70	70	163	173	135	134	0.57	0.75
70	67	148	130	100	159	1.08	1.82
72	59	158	146	88	25	1.48	2.76
66	63	130	130	82	65	1.23	1.67
73	71	146	130	69	42	1.76	2.25
Mean...70	66	149	142	95	65	1.22	1.85
							P < 0.04

* Fifteen gm. injected in 1 to 2 min., timed from start of injection.

† Assuming that the per cent per minute disappearance during a 30-min. period occurred at a constant rate.⁵

types of diabetic subjects is not known. In general, however, we have found in studies with carbutamide, tolbutamide, chlorpropamide, and metahexamide that there has been a good correlation between the potencies of the drugs in normal subjects and their potencies in diabetics of the maturity-onset type. However, this matter has not been systematically evaluated by us, and the opinion expressed here is based only on our "clinical impressions" and requires verification by more direct studies.

Summary and Conclusions

Chlorpropamide, 1 gm., produced significantly greater hypoglycemic responses in normal subjects 1 hour after ingestion than did tolbutamide, 1 gm. Chlorpropamide, 0.25 gm. and 0.5 gm., produced hypoglycemic responses in normal subjects 2 hours after ingestion comparable to doses of tolbutamide twice as great. Since it is unlikely that either drug had been appreciably metabolized or excreted 1 to 2 hours after ingestion, the relatively greater potency of chlorpropamide probably is not attributable entirely to its longer biological half time.

Seven normal subjects had serum chlorpropamide levels averaging 11.5 mg. per cent 2 hours after ingesting chlorpropamide, 1 gm., indicating that about one third of this dose was present in the plasma at that time.

Chlorpropamide, 0.5 gm., significantly increased the rate of disappearance of intravenously injected glucose. In one subject the effect of an intravenous injection of chlorpropamide, 0.5 gm., on the glucose disappearance rate was strikingly similar to that of 1.8 units of insulin administered intravenously.

Two findings that have resulted from studies carried out since this report was drafted are of considerable interest in connection with the initial findings. We found that serum levels of chlorpropamide two hours after the drug was administered were much higher than serum levels of tolbutamide after comparable doses of that drug. We found also that when chlorpropamide and tolbutamide were administered intravenously the hypoglycemic effects during the first hour were not significantly different.

Acknowledgments

The authors are indebted to B. J. Matter, Charles Key, Dorothy A. Wood, and Robert Henry for technical assistance in carrying out these studies.

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EFFECTS OF ADMINISTRATION OF CHLORPROPAMIDE UPON THE CARBOHYDRATE METABOLISM OF ISOLATED TISSUES*

D. W. Clarke

Department of Physiology, University of Toronto, Toronto, Ont., Canada

It has been shown previously that the administration of carbutamide to rats over a 14-day period can cause a slight increase in the level of glycogen in the diaphragm.¹ If this diaphragm is removed and incubated in a suitable medium there is a decrease in the glycogen level, and this decrease is greater in diaphragms from treated animals than in those from control animals. This larger drop is probably only a reflection of the higher initial level of glycogen, but the higher initial level is compatible with the theory that carbutamide acts by increasing the effective insulin level in the plasma, possibly by increasing insulin secretion by the pancreas. A similar amount of tolbutamide does not cause a statistically significant increase, although the experimental data suggest such an effect.

This type of experiment has been repeated, using chlorpropamide, to learn whether similar increases in muscle glycogen could be obtained. The drug was made up as either a 2½ per cent or a 1¼ per cent suspension in 1 per cent methyl cellulose (Methocel) solution and was force-fed daily to rats in measured amounts so that a dose of either 250 mg./kg./day, or 125 mg./kg./day was given. At the same time, Orinase† and RP 2259‡ were given to other rats in a dosage of 250 mg./kg./day. Control rats received only the Methocel. They were kept on this regime for from 5 to 7 days and were then sacrificed.

Results

The rats used in these tests were divided into a group of adult rats (Group 1) and a group of younger rats that were still growing (Group 2). The average weight changes are shown in the first two lines of TABLE 1. Generally speaking, the effect of the hypoglycemic agents is to cause a weight loss in the older animals or a decreased weight gain in the younger ones.

The results of measurements on initial glycogen levels in the diaphragm, on glycogen decrease in the diaphragm after incubation in a saline-phosphate medium, on glucose uptake of the diaphragm, on liver glycogen and glucose release by a liver slice, on plasma potassium, and on plasma glucose are further shown in TABLE 1. Results from the 2 groups of animals are pooled, since there seemed to be no difference between the younger and the older animals.

An analysis of the variance shows that there is a significant difference

* The work reported in this paper was supported in part by grants from Chas. Pfizer & Co., Inc., Brooklyn, N. Y., and from Poulenc, Ltd., Montreal, Canada.

† Tolbutamide.

‡ 2-Sulfamido-*p*-aminobenzene-5-*tert*.-butyl-1-thio-3, 4-diazole.

between the values for initial glycogen. Chlorpropamide (at its high level) and RP 2259 seemed to cause a small increase. Neither Orinase nor chlorpropamide (at its low dose level) show such an effect.

In a second experiment, chlorpropamide was given, 350 mg./kg./day, for 14 days. If anything, there was a drop in diaphragm glycogen level: 2.99 mg./gm. for controls, 2.77 mg./gm. for treated animals. The average weight gain for treated animals was 34 gm., compared to an average gain of 83 gm. for control animals. The test animals were in a poorer nutritional state, and this may account for their failure to show an increase in muscle glycogen.

An additional experiment in which Orinase and RP 2259 were given, 400 mg./kg./day, showed that after 3 days neither agent had any effect upon diaphragm glycogen, but that after 14 days the glycogen levels in diaphragms of rats treated with RP 2259 were 4.40 mg./gm., which is significantly greater than the control value of 1.93 mg./gm. Treatment with Orinase yielded a value of 2.04 mg./gm., which is not significantly greater than the control level.

TABLE 1

	Control	RP 2259	Orinase	Chlorpropamide		No. of animals
				1¼%	2½%	
Weight changes (grams)						
Group 1.....	6.3	-6.3	-2.7	-8.0	-7.7	3
Group 2.....	25.0	25.3	12.0	19.7	21.7	3
Initial diaphragm glycogen (mg./gm.).....	2.75	3.65	2.57	2.57	3.27	5
Glycogen decrease from original level (mg./gm.).....	1.30	1.20	1.02	0.96	1.49	5
Liver glycogen (mg./gm.)....	9.8	8.6	9.4	9.3	12.4	5
Liver glucose production (mg./gm.).....	11.9	10.5	9.2	10.3	8.3	5
Glucose uptake (mg./gm.)....	1.64	2.01	1.56	1.53	1.32	3
Blood glucose (mg. %).....	150	150	135	143	125 (162)	6
Plasma potassium (mEq./ml.)	6.18	6.11	6.37	6.45	5.99	4

The values for the first experiment (TABLE 1) show that there were no significant changes in glycogen losses in the diaphragm following incubation, in glucose uptake by the diaphragm, in liver glycogen or glucose production by the liver slice, or in plasma potassium levels. Blood sugar values show that there was a slight hypoglycemia in most cases. The value shown for the high dose of chlorpropamide has one very high value omitted. The average value, with this peculiar high value included, is shown in brackets. The experiments with the higher doses of the hypoglycemic agents showed that chlorpropamide caused a drop in liver glycogen to 57 per cent of the

control value. Orinase caused a drop to 42 per cent of the control value, where RP 2259 caused a drop to 60 per cent of the control value.

In Vitro Studies

The influence of chlorpropamide *in vitro* on certain tissues was also studied. The glucose output of liver slices from normally fed rats was measured when this compound was added to the incubation medium in different concentration. Slices were incubated in a Dubnoff shaker for 45 min. at 37° C. in 10 ml. of phosphate-saline buffer. The results are shown in TABLE 2.

TABLE 2

	Chlorpropamide concentration, mg./ml.			
	0.00	0.06	0.25	1.00
Glucose output mg./gm.	9.45	8.91	9.65	9.92
Per cent change from control.		-5.7	+2.1	+5.0

The inhibitory action of chlorpropamide on glucose output is evident at a concentration of the drug that is about the same as that reported in the blood of human subjects. This suggests that chlorpropamide may act, at least in part, by inhibition of liver activity. No further studies were done to elucidate the reason for the increase in glucose output with a higher concentration of chlorpropamide.

Chlorpropamide can also inhibit the oxygen uptake of certain tissue. Kidney slices from normal rats were incubated in a Krebs-Ringer phosphate medium in a Warburg apparatus, and oxygen utilization was measured. After 1 hour chlorpropamide was added, and it was found that at a concentration of 0.1 mg./ml. it gave a 19 per cent inhibition in the rate of oxygen uptake; at 0.01 mg./ml. it gave a 14 per cent inhibition.

When added to a rat liver homogenate at a concentration of 10^{-3} M (0.28 mg./ml.) in the presence of cytochrome *c* and succinate² there was a drop of 7 per cent in the oxygen consumption rate; at 10^{-2} M (2.8 mg./ml.) a drop of 13 per cent was observed.

Conclusion

Chlorpropamide may, under certain conditions, slightly increase the glycogen content of the rat diaphragm. Under similar conditions Orinase has no effect; RP 2259 has a greater effect. Any stimulating action upon glycogen synthesis is consistent with the hypothesis that certain oral hypoglycemic agents act by increasing the plasma insulin activity.

Chlorpropamide in low concentration can cause a small reduction in the glucose output of liver slices and a reduction in oxygen utilization by kidney slices and liver homogenates. It is suggested that part of the hypoglycemic action of chlorpropamide may come about through its hepatic action.

Acknowledgments

We are indebted to Chas. Pfizer & Co., Inc., for supplies of chlorpropamide and to Poulenc, Ltd. for supplies of RP 2259.

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IN VIVO EFFECT OF INSULIN AND CHLORPROPAMIDE ON CERTAIN LIVER ENZYMES IN RATS

Martin D. Sass

Medical Service, Veterans Administration Hospital, Brooklyn, N. Y.

Milton B. Handelsman

Medical Service, Veterans Administration Hospital, Long Island College Hospital, and State University of New York College of Medicine, Brooklyn, N. Y.

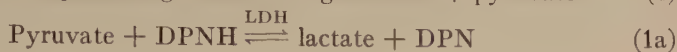
In the over-all animal economy, insulin is known to be protein sparing, a property that has not as yet been associated with the newer oral hypoglycemic agents. The mechanisms by which insulin exerts its effects on protein metabolism are still unclear, but it has been assumed that these effects are secondary manifestations of the alterations in carbohydrate metabolism known to occur in hypoinsulinism and hyperinsulinism. The present study was prompted by several reports^{1, 2} wherein it was proposed that the gluconeogenic action of the adrenal corticosteroids is based on their ability to stimulate the hepatic activity of glutamic-pyruvic transaminase (GPT), one of the two major transaminase systems involved in protein metabolism. The effect of insulin and chlorpropamide on GPT and glutamic-oxaloacetic transaminase (GOT) activity seemed worthy of study. The results presented below compare the effects of these two hypoglycemic agents on GPT and GOT activity with those of hydrocortisone. Lactic dehydrogenase (LDH) activity was also studied as an example of an enzyme system not directly concerned with protein metabolism.

Experimental

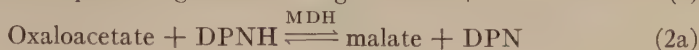
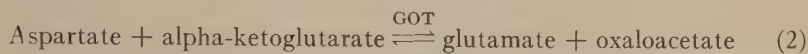
Eighty-two albino rats weighing from 100 to 200 gm. were used for this study. Food and water were offered freely unless otherwise indicated. Protamine zinc insulin and chlorpropamide (sodium salt) were administered in varying doses of from 1 to 4 U./kg. and from 50 to 100 mg./kg., respectively. Hydrocortisone acetate was administered in a dosage of 5 mg. per animal. With the exception of one preliminary acute experiment, all drugs were given subcutaneously in 7 daily doses over an 8-day period.

On the day of sacrifice all food and drugs were withheld. The animals were stunned and exsanguinated. The livers were removed immediately and portions were weighed for dry-weight and enzyme assays. Those portions removed for assay were immediately frozen in a dry ice-alcohol bath and stored at -20° C. prior to analysis. A 5 per cent homogenate of the sample for assay was prepared in the cold, and suitable dilutions for each assay were prepared.

GPT activity was measured by a modification of the method of Wróblewski.³ In this determination pyruvic acid formed in REACTION 1 is reduced to lactate after the addition of excess LDH and in the presence of reduced diphosphopyridine nucleotide (DPNH).



GOT activity was measured by a modification of Karmen's procedure.⁴ In this assay oxaloacetate formed by REACTION 2 is reduced to malate by an excess of malic dehydrogenase (MDH) and in the presence of DPNH as in REACTION 2a.



LDH activity was determined by a modification of a procedure based on REACTION 1a.⁵ The oxidation of DPNH in each of these reactions was followed spectrophotometrically at 340 m μ . One unit of activity for all enzymes represents a change in optical density of 0.001 O.D. units/min. Although all reactions were carried out within a fairly narrow temperature range, the results were corrected to a temperature of 25° C. by a separately determined series of temperature-correction factors, a procedure found desirable in previous studies.^{6, 7}

Aliquots of the original homogenates were analyzed for nitrogen by the micro-Kjeldahl method. Although not always presented, results were calculated in terms of wet weight, dry weight, and nitrogen content.

Results

Preliminary experiments were performed to ascertain the acute effect of the administration of insulin and chlorpropamide on liver enzyme activity. Three pairs of rats were fasted for 18 hours before use. Crystalline insulin (2 U./kg.) was administered subcutaneously to one pair at zero and 1½ hours. A second pair received one injection of chlorpropamide (100 mg./kg.) at zero hours. Both pairs were sacrificed at 3 hours, together with a third pair that served as a control. No significant differences in hepatic enzyme activity were found between the control and treated animals (TABLE 1) despite markedly depressed terminal blood sugars in the latter groups.

The absence of any change in this acute experiment is in line with what has been found in studies concerning the acute effects of insulin administration.⁸ Hastings *et al.*⁹ have concluded that insulin exerts immediate metabolic effects only at the periphery, and that its effect on hepatic metabolism is delayed and gradual. Further experiments reported herein were all extended to eight days.

Effects of insulin, chlorpropamide, and hydrocortisone in rats fed ad libitum. TABLE 2 presents the results of one experiment evaluating the effect of insulin (1 U./kg./day) and chlorpropamide (50 mg./kg./day) given in 7 injections over an 8-day period. The actual results for each animal are illustrated here to demonstrate the variability of individual measurements and to compare the results on the basis of wet weight, dry weight, or nitrogen

TABLE 1
EFFECT OF ACUTE ADMINISTRATION OF INSULIN AND CHLORPROPAMIDE ON HEPATIC
ENZYME ACTIVITY

Treatment	Enzyme activity U./mg. dry wt.		
	GPT	GOT	LDH
(a) No treatment.....	84.4	973	1420
(b) Insulin (regular), 4 U./kg.....	83.5	1000	1450
(c) Chlorpropamide (Na), 100 mg./kg.....	78.8	910	1315

TABLE 2
VARIABILITY IN HEPATIC ENZYME ACTIVITY IN RATS

Treatment	Enzyme activity (units)								
	GPT			GOT			LDH		
	1*	2*	3*	1	2	3	1	2	3
(a) Control	19.3	64.7	609	213	715	6720	442	1480	13,900
	21.5	72.3	658	203	633	6230	438	1470	13,400
	23.1	74.5	663	237	764	6790	403	1300	11,600
Mean	21.3	70.5	645	218	704	6580	428	1417	13,000
(b) Insulin (PZI) 1 U./kg.	12.5	39.5	359	211	666	6063	342	1080	9,827
	15.6	46.3	414	206	612	5464	399	1180	10,600
	15.6	48.1	405	207	648	5370	381	1170	9,900
Mean	14.6	44.6	393	208	642	5630	374	1140	10,100
(c) Chlorpropamide 100 mg./kg.	19.3	58.8	553	197	601	5640	305	930	8,740
	16.9	54.0	469	199	636	5530	356	1140	9,900
	15.8	51.3	451	152	494	4350	419	1360	12,000
Mean	17.3	54.7	491	183	577	5180	360	1140	10,200

* Columns 1, 2, and 3 represent units/mg. wet weight, dry weight, and nitrogen, respectively.

content. Final evaluation of the effects of therapy were made on the combined data from this and subsequent experiments on 27 animals.

The results, shown in TABLE 3a indicate that hydrocortisone has a differing action on the 2 hepatic transaminases studied. Thus, although steroid administration apparently had no effect on GOT activity, it resulted in a marked and significant ($p < 0.01$) increase in GPT activity. These latter

TABLE 3
EFFECT OF THERAPY ON HEPATIC ENZYME ACTIVITY IN FED AND RESTRICTED ANIMALS

Treatment	Enzyme activity (\pm S.E.) U./mg. dry wt.		
	GPT	GOT	LDH
(a) Ad libitum feeding			
Control.....	67.4 (± 1.9)	701 (± 10)	1300 (± 25)
Chlorpropamide, 50-100 mg./kg.....	55.8 (± 1.8)	565 (± 5.3)*	1090 (± 13)
Insulin (PZI), 1-4 U./kg.....	56.2 (± 1.7)	572 (± 7.1)*	1200 (± 16)
Hydrocortisone, 5 mg.....	181 (± 8.6)*	689 (± 36)	1430 (± 88)
(b) Ad libitum feeding plus hydrocortisone			
Hydrocortisone alone.....	181 (± 8.6)*	689 (± 36)	1430 (± 88)
Hydrocortisone plus chlorpropamide, 100 mg./kg.....	214 (± 4.2)*	584 (± 12)*	1200 (± 21)
Hydrocortisone plus insulin (PZI), 2 U./kg.....	195 (± 7.6)*	524 (± 33)*	1170 (± 29)
(c) Restricted feeding			
No treatment.....	125 (± 2.8)*	976 (± 22)	966 (± 14)*
Hydrocortisone.....	197 (± 3.7)*	755 (± 15)*	1090 (± 29)
Chlorpropamide, 100 mg./kg.....	101 (± 2.8)*	857 (± 22)	1130 (± 37)
Insulin (PZI), 2 U./kg.....	110 (± 4.3)*	923 (± 31)	984 (± 35)*

* Significantly different from control values ($p < 0.01$).

findings are in accord with numerous other reports on the effect of adrenocorticosteroids on the glutamic-pyruvic transaminase.^{1, 2, 10, 11} Although GOT activity has been reported to be unaffected by similar therapy,^{1, 11, 12} in line with our present findings, there have been conflicting reports.^{2, 13} This disagreement, however, may be a matter of degree, since it is the consensus that GOT activity is much less markedly influenced by steroids, if at all, than is GPT.

In contrast to these findings, the administration of insulin (1 to 4 U./kg./day) and chlorpropamide (50 to 100 mg./kg./day) were both shown to be accompanied by a significant decrease ($p < 0.01$) in hepatic GOT activity, as shown in TABLE 3a. GPT activity, although apparently somewhat depressed, was not significantly affected ($p > 0.18$). No reports of similar *in vivo* studies on the effects of insulin or chlorpropamide on hepatic transaminases have come to our attention. However, our observations would

seem to be in accord with the report by Copenhaver *et al.*¹⁴ that hepatic GOT activity is markedly elevated in alloxan-diabetic rats.

Lactic dehydrogenase catalyzes a reaction that is intimately connected with glycolysis and the tricarboxylic acid cycle. In addition, it shares a common substrate, pyruvic acid, with the glutamic-pyruvate transaminase system. The finding, shown in TABLE 3a, that this enzyme remained unchanged in all of the groups studied was surprising. This fact, together with the differing action of hydrocortisone and insulin (and of chlorpropamide), suggests that the changes in transaminase activity observed in these experiments are more than mere nonspecific reflections of a drug-altered liver biochemistry.

Effect of insulin and chlorpropamide in rats fed ad libitum and given hydrocortisone. The question arose as to whether the effect of both insulin and chlorpropamide on GOT activity was due to a direct hormonal influence on the liver or an indirect type of response due to changes in the periphery. Hydrocortisone, together with either insulin or chlorpropamide, was administered to a group of 17 animals to compare the effects of a combination of these drugs with the action of each alone.

It can be seen in TABLE 3b that the administration of insulin (2 U./kg./day) or chlorpropamide (100 mg./kg./day) to hydrocortisone-treated rats (5 mg./day) resulted in changes in enzyme activity that reflected the influence of the steroid as well as the hypoglycemic agents. Thus, GPT activity was increased as a result of hydrocortisone therapy despite the simultaneous administration of the hypoglycemic agents. As shown in TABLE 3a, GOT activity was depressed after the injection of insulin or chlorpropamide, despite the administration of hydrocortisone. Lactic dehydrogenase activity again remained unaffected.

Effect of insulin and chlorpropamide in rats fed a restricted diet. Starvation over a period of 4 days has already been shown to augment the hepatic activity of both glutamic-oxaloacetic and pyruvic transaminase.¹³ The effect of hydrocortisone, insulin, and chlorpropamide on this increase in enzyme activity was studied in a group of thirty-two animals.

TABLE 3c shows that even the limited food restriction imposed in the present experiments was capable of enhancing the activity of the two transaminases under consideration. The administration of hydrocortisone to these animals resulted in a further augmentation of GPT activity beyond that produced by food limitation alone. GOT activity, on the other hand, was apparently decreased to those levels encountered in ad libitum-fed animals. These findings further substantiate previous indications that hydrocortisone has a diverse effect on these two closely related enzymes. Contrary to the findings with animals on a free diet, neither insulin nor chlorpropamide could be shown to exert any influence on the activity of these enzymes beyond that associated with food limitation.

TABLE 3c shows that the level of LDH activity was once again unaffected by the administration of the three drugs studied. Food restriction alone, however, resulted in a significant decrease ($p < 0.01$) in the level of this enzyme in contrast to the increases observed in the level of both transaminases. It is interesting that limitation of dietary intake was the only

experimental procedure capable of altering the activity of the lactic dehydrogenase enzyme.

Discussion

The results obtained in this investigation must be interpreted with caution. Much additional work is needed to elucidate the role that these enzymes play in whole body homeostasis. One fact stands out: namely, that the action of chlorpropamide paralleled that of insulin in all experiments. The cause and physiological significance of these changes in hepatic enzymes, however, are more difficult to evaluate.

The early work of Bach and Holmes¹⁵ showed that the addition of insulin to liver slices of normal fasted rats inhibited the transformation of certain amino acids into carbohydrates. Jensen and Gray,¹⁶ employing both *in vitro* and *in vivo* techniques, found that insulin exerts an inhibitory effect on hepatic amino acid dehydrogenase activity in normal rats. In their *in vivo* experiments insulin administration led to a 25 per cent decrease, and adrenal cortical extracts to a 205 per cent increase in the activity of this enzyme. Since Stadie *et al.*¹⁷ showed that the enzyme influenced by insulin affects only the unnatural D-isomer of amino acids, the physiological importance of these findings is doubtful. However, it is interesting to note that a similar increase in GPT and a decrease in GOT activity were obtained in this study after hydrocortisone and insulin, respectively. This similarity would appear to throw some doubt on the view that the change in transaminase activity after steroid therapy is a reflection of specific hormonal control.

This latter concept was suggested by Rosen *et al.*¹ as an explanation of the marked increase in GPT activity and subsequent gluconeogenesis accompanying the administration of cortisone. The increase in enzyme activity after food restriction might be construed as a reflection of a stress-induced oversecretion from the adrenal cortex. The differences in the action of insulin and chlorpropamide under the conditions of restricted and unrestricted feeding make this interpretation unlikely.

Starvation, however, like hydrocortisone therapy, is accompanied by an alteration in the metabolism of proteins toward catabolism. Insulin appears to stimulate anabolism. It has already been suggested that hepatic transaminase activity is inversely related to protein synthesis,^{10, 18} a view that appears to accord with current findings. Similarly, Recant and Fischer¹⁹ recently presented data indicating that tolbutamide, as well as insulin, stimulates the incorporation of tagged glycine into the liver proteins of starved animals. These investigators, however, believe that this represents an effect of a more adequate utilization of carbohydrates.

It is, of course, impossible to determine at present whether these observed changes in enzyme activity are under direct hormonal control or whether they represent secondary manifestations of a substrate-induced enzyme adaptation.²⁰ It is even difficult to be sure that they represent actual changes in the processes catalyzed by these enzymes. This fact has been emphasized by the findings of Zuchlewski and Gaebler,²¹ who demonstrated that the transfer of isotopic nitrogen from alanine to other amino acids occurred to the same

extent in the livers of normal and hypophysectomized rats treated with growth hormone, despite a marked reduction in the GPT activity of the hormone-treated animals. Similar studies on the effects of hydrocortisone, insulin, and chlorpropamide might yield a more definitive concept of the mechanisms by which these play a role in protein metabolism and its relation to gluconeogenesis.

Summary

Insulin and chlorpropamide act similarly in moderately depressing glutamic-oxaloacetic transaminase activity in the livers of well-fed animals. The simultaneous administration of hydrocortisone appears to have no effect on this action of insulin and chlorpropamide.

Glutamic-pyruvic transaminase activity, augmented by the administration of hydrocortisone alone, continued to be markedly increased despite the simultaneous administration of insulin and chlorpropamide.

Food restriction increased the activity of both transaminases. Under these conditions, neither insulin nor chlorpropamide could be shown to exert any effect on GOT activity despite the further augmentation of GPT activity by hydrocortisone.

Further study is necessary to determine whether these changes in transaminase activity induced by these drugs are secondary in nature or whether they represent direct hormonal control.

Acknowledgment

We acknowledge the valuable technical assistance we received from Carmine J. Caruso in the experiments reported in this paper.

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SOME EFFECTS OF TOLBUTAMIDE AND CHLORPROPAMIDE IN VITRO*

Albert E. Renold, Gaston R. Zahnd,† Bernard Jeanrenaud,‡
Buris R. Boshell

*Baker Clinic Research Laboratory and the Departments of Medicine, Harvard Medical School
and Peter Bent Brigham Hospital, Boston, Mass.*

Introduction

Although a detailed discussion of available information concerning the site of action of the hypoglycemic arylsulfonylureas would seem superfluous at this time (particularly since this is a matter still subject to interpretation), I propose to outline briefly our group's present point of view. We presently consider well documented the position that the acute administration of the arylsulfonylureas leads to a decrease in the pancreatic insulin content¹ and to an increase in the insulinlike activity of pancreatic vein blood.²⁻⁴ Increased circulating insulinlike activity, however, has not as yet been established in man.^{5, 6} Whether the same sequence of events holds true during long-term, chronic administration of these compounds is as yet unknown, as is the relative physiological importance of the postulated increased insulin secretion—either acutely or during long-term administration. We also consider as well established the view that the hypoglycemic sulfonylureas exert direct, extrapancreatic metabolic effects; at the same time, we fully acknowledge that, again, the relative physiological importance of these effects is unknown and—mildly stated—disputed.

The group for which I am speaking, associated with George W. Thorn's Metabolic Unit at Harvard Medical School and the Peter Bent Brigham Hospital, was impressed early by the existence of significant differences in the metabolic states induced by insulin and the sulfonylureas, respectively, even when the severity of the induced hypoglycemia was of similar magnitude and achieved in a similar period of time.^{5, 7} If one tends to accept the insulin-stimulation theory of sulfonylurea action as the major site of action of these substances, this paradox may be resolved by endowing endogenous insulin secreted into the portal vein with special, previously unknown properties.⁸ If, on the other hand, one is not particularly impressed by the evidence gathered for the over-all physiological importance of the insulin stimulation theory of sulfonylurea action, one is led logically to give prominence to the extrapancreatic effects of the compounds. Our group still favors the latter interpretation, but I stress the fact that the following observations are not presented as additional evidence for or against the physiological importance of sulfonylurea-induced insulin secretion. Since heated discussion on this

* Research reported in this paper was supported by grants from Chas. Pfizer & Co., Inc., Brooklyn, N. Y., and The Upjohn Company, Kalamazoo, Mich.

† Recipient of a Research Fellowship from the Swiss Academy of Medical Sciences, Basel, Switzerland.

‡ Recipient of a Research Fellowship from the Dazian Foundation for Medical Research, New York, N. Y.

point seems rather fruitless at present, it is hoped that the effects shown will contribute to the over-all pharmacodynamic description of these interesting agents. Indeed, it may be permissible to expect that careful analysis of the extrapancreatic effects of the sulfonylureas, whatever their physiological significance, may lead as well to the description of a mechanism for the sulfonylurea-induced release of insulin.⁹

Material and Methods

We are here concerned exclusively with the effects of tolbutamide and chlorpropamide on ketogenesis by liver slices from rats fasted 48 hours, and with the drugs' effects on glucose metabolism by adipose tissue from fed, normal rats. The methodology employed has been described previously in some detail.^{10, 11} Alloxan diabetes was induced by the intravenous injection of 40 mg./kg. alloxan following a 48-hour fast. Random blood glucose values exceeding 350 mg. per cent within 2 or more weeks following alloxan injection were considered as indicative of the presence of diabetes. Total pancreatectomies were performed according to Scow,¹² and only those animals exhibiting both hyperglycemia and ketosis 36 hours following pancreatectomy were used.

Results

Buris Boshell of our laboratory has reported¹⁰ that tolbutamide in concentrations of 1.0 mg./ml. and 0.2 mg./ml. significantly inhibits the formation of ketones by rat liver slices *in vitro*. The previously reported data are shown in TABLE 1. It was considered of particular significance that this

TABLE 1*
EFFECT OF TOLBUTAMIDE UPON KETOGENESIS BY LIVER SLICES FROM NORMAL RATS FASTED 48 HOURS

Addition to medium	No. of observations	Ketogenesis <i>in vitro</i>	
		Micrograms acetone-equivalent/ gm. wet liver/ 90 min.	Control (percentages)
No addition.....	7	610 ± 63†	100
Insulin, 0.1 U./ml.....	7	590	97 ± 4†
Tolbutamide, 1.0 mg./ml.....	7	226	38 ± 4
No addition.....	8	539 ± 100	100
Tolbutamide, 0.2 mg./ml.....	8	376	70 ± 6

* Data from Boshell *et al.*¹⁰ Incubations were carried out in Krebs bicarbonate buffer modified by replacing some Na⁺ by K⁺ to final concentrations of Na⁺ = 83 and K⁺ = 60 mEq./l.

† Mean ± standard error of the mean. Since all experiments were *paired* experiments, the significance of the tolbutamide effect is calculated on the basis of the figures representing percentage of the control value.

tolbutamide effect could be demonstrated at a concentration (0.2 mg./ml. or 20 mg. per cent) quite frequently encountered in plasma or serum of tolbutamide-treated patients, and that the addition of insulin at high concentrations was ineffective in the same system, thus precluding an interpretation of insulin mediation of the tolbutamide effect.

These observations have since been extended to include a greater range of concentrations and the comparison of chlorpropamide effects with the effects of tolbutamide. As shown in FIGURE 1, tolbutamide and chlorpropamide were approximately equally effective in depressing *in vitro* ketogenesis by liver slices from rats fasted 48 hours, although the data do not exclude

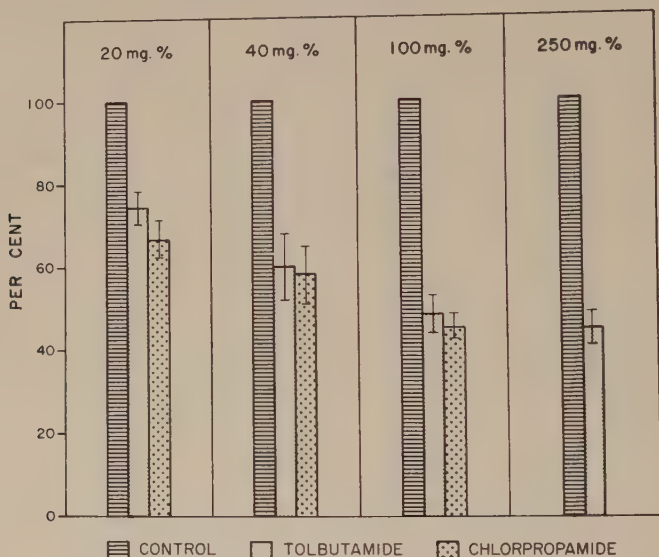


FIGURE 1. Effect of tolbutamide and chlorpropamide on ketogenesis by liver slices from rats fasted 48 hours. Top figures refer to the concentrations of drugs used, expressed as milligrams per 100 ml. Incubations were carried out in a Krebs bicarbonate buffer modified by adjusting the concentrations of K^+ to 60 and Na^+ to 83 mEq./l.

the possibility that a differential potency of the two substances might have been detected at lower concentrations (for example, 10 mg. per cent). The absence of a striking potency difference with regard to ketogenesis *in vitro* thus tentatively supports the view that *in vivo* potency differences of the 2 compounds are more likely to be related to differences in the metabolic fate of the drugs rather than to differences in their intrinsic metabolic activities.

FIGURE 2 further demonstrates the effects of tolbutamide and chlorpropamide upon hepatic ketogenesis as a function of time. It is evident that the effect appears early and persists throughout the period of incubation. The figure also demonstrates that the over-all drug-induced inhibition is really greater than that previously estimated, since a major portion of the ketones present in the incubation medium (at the end of the incubation in the presence of tolbutamide and chlorpropamide) represents ketones

already present in the liver at the time of slicing. Furthermore, sulfanilamide and, in other experiments, sulfaguanidine (both nonhypoglycemic or, at least, less hypoglycemic) exhibited much less or no antiketogenic activity *in vitro*.

Whereas correlation between hypoglycemic activity *in vivo* and antiketogenic activity *in vitro* is at least consistent with the possibility of a common mechanism of action, further studies carried out in liver slices obtained from animals in which experimental diabetes had been induced by alloxan injection or by pancreatectomy failed to support this conclusion. As shown in FIGURE 3, tolbutamide effectively suppressed hepatic ketogenesis in the

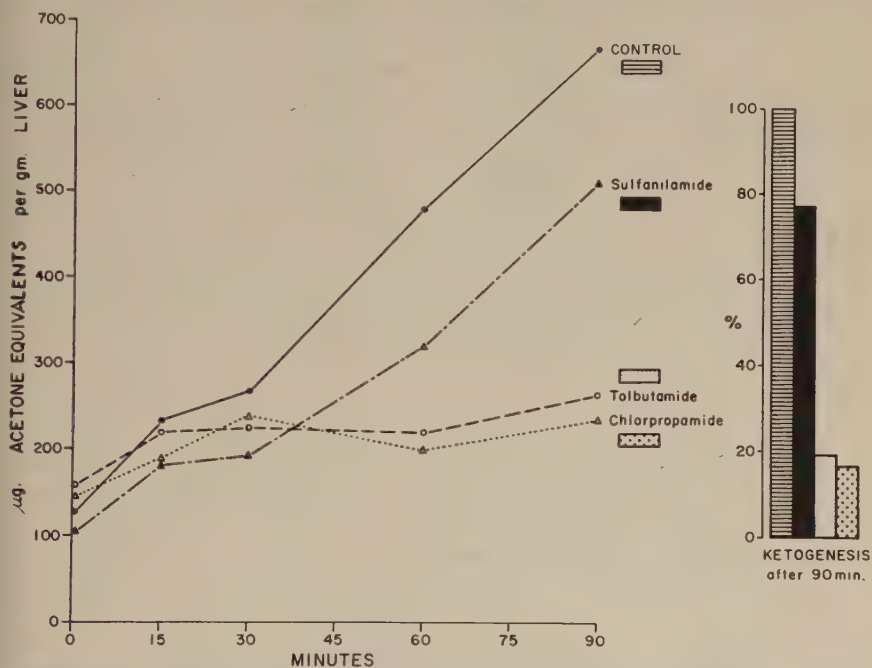


FIGURE 2. Effect of tolbutamide (100 mg. per cent), chlorpropamide (100 mg. per cent), and sulfanilamide (100 mg. per cent) on ketogenesis by liver slices from rats fasted 48 hours. Each curve represents the mean of 2 experiments.

presence of alloxan diabetes or pancreatectomy, while chlorpropamide similarly depressed ketogenesis of liver slices obtained from pancreatectomized rats. Since the hypoglycemic activity of tolbutamide or chlorpropamide *in vivo* is greatly decreased in animals so treated, a discrepancy appears to exist here between the effects observed *in vitro* and *in vivo*.

Prod'Hom and Plattner,¹³ in Favarger's laboratory in Geneva, Switzerland, have recently reported a striking inhibition of lipogenesis by mouse adipose tissue, both *in vivo* and *in vitro*, resulting from the administration of carbutamide for several days. The dosage used, however, was large. Ashmore and his collaborators,¹⁴ working with rats, observed increased hepatic lipogenesis and relatively unchanged adipose tissue lipogenesis following the

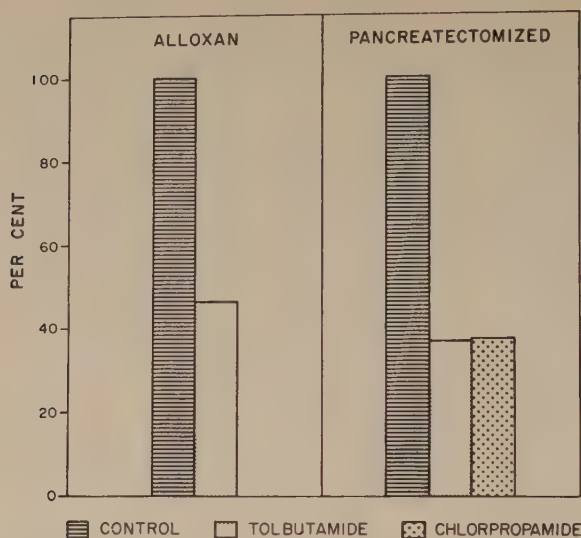


FIGURE 3. Effect of tolbutamide and chlorpropamide (100 mg. per cent) on ketogenesis by liver slices from alloxan-diabetic and pancreatectomized rats. Shown are the means of four (alloxan) and six (pancreatectomized) experiments.

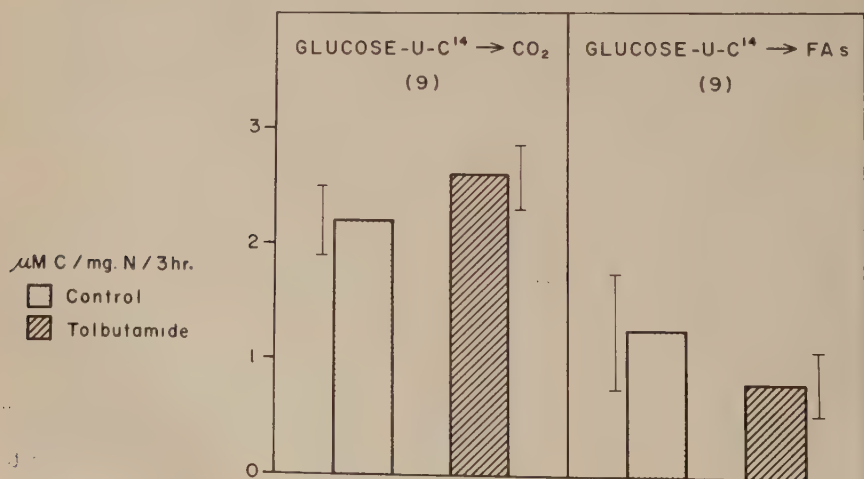


FIGURE 4. Effects of tolbutamide (0.8 mg./ml.) upon rat adipose tissue *in vitro*. The standard errors shown do not take into account the paired nature of the data. The number of experiments is shown in parentheses. FA represents fatty acids.

Tolbutamide effect: CO₂ = +0.34 ± 0.16

Fatty acids = -0.46 ± 0.25

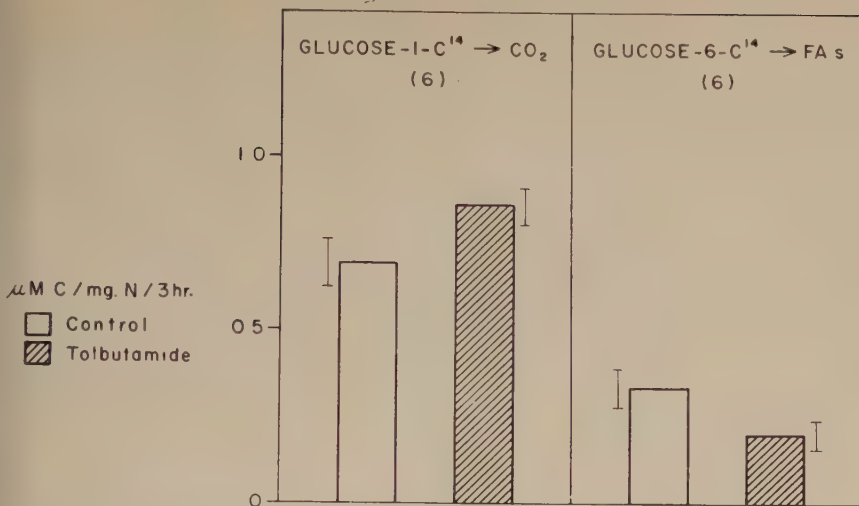


FIGURE 5. Effects of tolbutamide (0.8 mg./ml.) upon rat adipose tissue *in vitro*. The standard errors shown do not take into account the paired nature of the data. The number of experiments is shown in parentheses. FA represents fatty acids.

Tolbutamide effect: CO₂ = +0.17 ± 0.07
Fatty acids = -0.13 ± 0.05

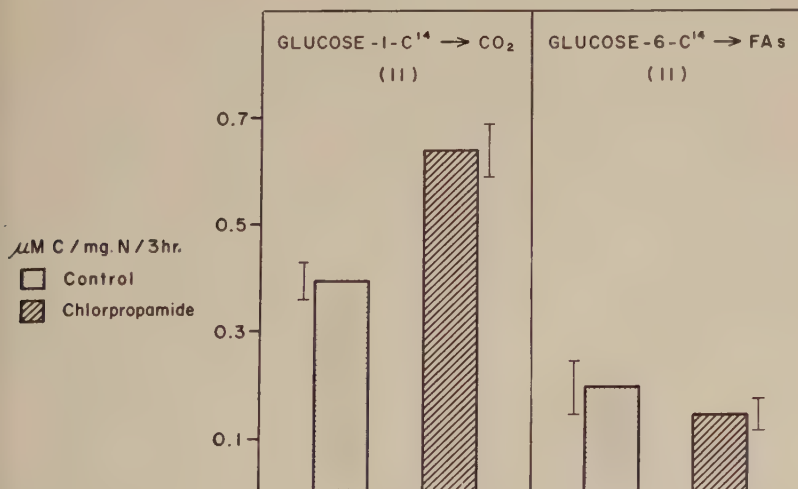


FIGURE 6. Effects of chlorpropamide (0.8 mg./ml.) upon rat adipose tissue *in vitro*. The standard errors do not take into account the paired nature of the data. The number of experiments is shown in parentheses. FA represents fatty acids.

Chlorpropamide effect: CO₂ = +0.21 ± 0.03
Fatty acids = -0.05 ± 0.02

acute administration of tolbutamide. Recently, we investigated the effects of tolbutamide upon the metabolism of glucose by rat adipose tissue and observed (FIGURE 4) that the presence of tolbutamide (0.8 mg./ml.) resulted in increased CO_2 production from uniformly labeled glucose, but in decreased lipogenesis from the same substrate. This combination of effects seemed rather unusual since, as a rule, increased glucose oxidation is also accompanied by increased synthesis of fatty acids from glucose carbon.^{11, 15, 16} When the observations were repeated with glucose-1- C^{14} and glucose-6- C^{14} as substrates (FIGURE 5), it appeared that the major contributor to the increased CO_2 production from glucose was carbon-1, suggesting stimulation of the phosphogluconate-oxidative pathway,¹⁷ while lipogenesis, again, was impaired. Finally, FIGURE 6 demonstrates a clear-cut stimulation of the oxidation of carbon-1 of glucose to CO_2 in the presence of chlorpropamide (0.8 mg./ml.), without concurrent stimulation of lipogenesis and, indeed, with a small inhibition of lipogenesis from glucose-6- C^{14} .

Discussion

The observations described simply add two further extrapancreatic effects of the arylsulfonylureas to those already reported on the rat hemidiaphragm,^{18, 19} on a number of hepatic enzymes related to glycolysis or gluconeogenesis,^{20, 21} on epinephrine-induced glycogenolysis,²² on hepatic transaminase activity,²³ and on a number of DPN- or TPN-dependent reactions.⁹ Their importance in explaining the hypoglycemic effects of the compounds is unknown, as is the mechanism of the antiketogenic effect described. Preliminary studies have failed to demonstrate increased CO_2 production or increased fatty acid synthesis from acetate associated with decreased ketogenesis, in the presence of acetate-1- C^{14} as the substrate. Neither the synthesis of cholesterol nor the activity of the reactions leading to the production of acetoacetate from acetoacetyl-coenzyme A, however, has as yet been measured under these conditions. It may be of some interest to point out that the observations relating to glucose oxidation and to lipogenesis from glucose by adipose tissue are in accord with the only attempt as yet made to find a common denominator for most or many effects of the arylsulfonylureas on enzyme systems. Wallenfels *et al.*⁹ pointed out that the enzymatic reactions affected by these compounds are frequently dependent upon pyridine nucleotides as cofactors. Indeed, they have even suggested a hypothetical mechanism of insulin release on this basis. The combination of increased CO_2 production from carbon-1 of glucose with decreased fatty acid synthesis is compatible with the presence of some interference in the transfer of hydrogen from glucose-6-phosphate to fatty acids by way of reduced TPN. A similar mechanism has been postulated by Cahill and his collaborators²⁴ for the effects of methylene blue and certain other hydrogen acceptors in liver slices.

Summary

Two extrapancreatic metabolic effects of tolbutamide and chlorpropamide have been described. First, both substances have been shown to inhibit

ketogenesis by liver slices from rats fasted forty-eight hours at concentrations within the expected therapeutic range. This effect is also present when the liver slices have been obtained from alloxan-diabetic or pancreatectomized animals. The mechanism by which this antiketogenesis is brought about is unknown. Second, both substances affect glucose oxidation and lipogenesis from glucose by surviving rat adipose tissue in a manner suggesting interference with the proper transfer of hydrogen from glucose to the fatty acid chain, perhaps at the level of the transferring pyridine nucleotides. The second observation is in accord with the generalization proposed by Wallenfels for a possible mechanism of action of the arylsulfonylureas upon tissues. The relevance of the two effects described to the hypoglycemic action of the arylsulfonylureas in man is unknown.

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Part II. Metabolic Studies

INHIBITION OF THE DIABETOGENIC ACTION OF SOMATOTROPIN BY TOLBUTAMIDE*

I. Arthur Mirsky, Simon Gitelson, Gladys Perisutti

*Department of Clinical Science, University of Pittsburgh School of Medicine,
Pittsburgh, Pa.*

The mechanism whereby the daily administration of diabetogenic extracts of the anterior pituitary gland or of purified growth hormone (somatotropin) to the dog results in the development of a temporary diabetic state (idiophypophyseal diabetes) that is followed by a permanent diabetic state (metahypophyseal diabetes) is still unknown. A good deal of evidence can be marshalled in support of the hypothesis that somatotropin induces an increased peripheral need for insulin, with a resultant increase in the production of insulin by the beta cells of the pancreas.¹⁻³ Accordingly, it has been proposed that when the rate of insulin utilization exceeds the rate at which it is manufactured, the beta cells are stimulated excessively and undergo degranulation, hydropic degeneration, and atrophy. Thus, the idiophypophyseal diabetes is attributed to a relative insufficiency in the capacity of the pancreas to meet the increased peripheral requirements for insulin, while the metahypophyseal diabetes that persists after the cessation of the somatotropin injections is attributed to the exhaustion, degeneration, and destruction of the beta cells.¹⁻⁶

The same sequence of morphologic changes that is observed in the islet tissue of the somatotropin-treated dog⁷ is found also during the development of diabetes in the partially depancreatized cat⁸ and dog⁹ and in the normal or partially depancreatized dog or cat, which develops a metadiabetes after the administration of thyroid extracts,¹⁰ anterior pituitary extracts,^{4-6, 11, 12} adrenal corticosteroids,¹³ or glucose.¹⁴ Degranulation and various degrees of hydropic degeneration of the beta cells are found also in animals with the idiadiabetes produced during the course of the administration of anterior pituitary extracts or somatotropin,^{7, 11, 12} corticosteroids,¹⁵⁻¹⁷ and large quantities of glucose.¹⁸⁻²⁰ Concomitant with the degranulation and hydropic degeneration is a reduction in the concentration of extractable insulin in the pancreas.^{7, 12, 21, 22}

In accord with the hypothesis that an excessive demand for insulin is a determinant in the production of metadiabetes are the observations that regimes that reduce the peripheral requirements for insulin may also reduce the severity of the idiadiabetes and prevent the development of the metadiabetes produced by various diabetogenic agents.²³ Fasting, undernutrition, a high-fat diet, and treatment with insulin appear to reduce the require-

* The work reported in this paper was supported in part by Grant A-1409 from the National Institute of Arthritis and Metabolic Diseases, Public Health Service, Bethesda, Md.

ments for insulin and thereby reduce the production of insulin by the pancreas.²⁴⁻²⁷ These measures inhibit the effect of diabetogenic factors in varying degrees. Thus, the changes in the morphology of the islets of Langerhans and the metadiabetes that may occur in partially depancreatized animals are inhibited or corrected by undernutrition²⁸ and by the administration of insulin.²⁹ Likewise, the diabetogenic action of anterior pituitary preparations is diminished or inhibited by undernutrition, high fat diets, and the administration of insulin.^{11, 12, 23, 30}

Although it is generally agreed that the degranulation of the beta cells produced by diabetogenic agents is due to excessive stimulation of the production and/or release of insulin by the beta cells, the significance of the hydropic degeneration in the development of metadiabetes remains unknown. The observations of Toreson³¹ and of Duff and Toreson³² established that the morphologic changes identified as "hydropic degeneration" reflect the deposition of large amounts of glycogen in the beta cells. These studies were extended by Lazarus *et al.*,^{17, 33} who employed a variety of diabetogenic agents and concluded that hydropic degeneration is not due to exhaustion of the beta cells, but is a facet of the increased glycogen deposition in cells, such as occurs in the kidney and heart of the diabetic animal. These studies, however, do not negate the hypothesis that excessive stimulation of the beta cells is responsible for their exhaustion and atrophy.

Irrespective of the precise mechanism whereby metadiabetes is produced by somatotropin, it is now quite evident that stimulation of the beta cells and the release of insulin into the circulation is an early effect of its action.¹⁻³ Thus, the administration of purified somatotropin (2 to 3.5 mg./kg./day to dogs for 6 to 8 days results in an extensive degranulation of the beta cells and a reduction in the concentration of extractable insulin in the pancreas; this may occur even before the development of hyperglycemia.⁷ The extent and the rapidity with which the degranulation occurs is similar to that produced by a number of agents that do not produce a permanent diabetes.³³ Among the latter are the arylsulfonylureas.

Soon after the discovery of the hypoglycemic action of isopropylthio-diazole,³⁴ Loubatières³⁵ established that the action of this and related sulfonamide derivatives is dependent upon the presence of more than one tenth to one fifth of the pancreas. Since the introduction of carbutamide and tolbutamide as orally effective hypoglycemic agents it has been firmly established that their hypoglycemic action is dependent upon the presence of islet tissue capable of producing insulin. Although it is quite probable that a number of mechanisms are involved in the action of these agents, there is ample evidence that one of these mechanisms is the stimulation of the beta cells and the release of insulin from pancreatic stores.^{35, 36}

Of particular interest are the observations that the administration of sulfonylureas to rats, rabbits, and dogs results in extensive degranulation of the beta cells³⁷⁻⁴¹ and a decrease in the concentration of extractable insulin in the pancreas.⁴² That these changes in the islets are associated with physiological effects that are somewhat similar to those produced during the early stages of the administration of somatotropin is suggested by the dimin-

ished glucose tolerance that occurs in rats and dogs treated with carbutamide for a prolonged period.³⁷ In accord also is the demonstration that the usual hypoglycemic phase of the response to the injection of alloxan does not occur in rats given large doses of tolbutamide for 3 or more days.⁴¹

Although some of the early effects of somatotropin appear to be similar to those produced by the sulfonylureas, the mechanisms involved appear to be quite different. Whereas the excessive stimulation of the beta cells that occurs in the dog treated with somatotropin is probably secondary to an increase in the peripheral requirements for insulin, that resulting from the administration of the sulfonylureas is probably due to a direct effect on the islets. Thus, the insulin requirements of the completely depancreatized dog are increased by somatotropin^{1-3, 43} and not by the sulfonylureas.⁴⁴⁻⁴⁸ Nevertheless, the similarity in the morphologic changes produced by these agents prompted a study on the effect of somatotropin on the acute hypoglycemic action of a single injection of tolbutamide and on the effect of the daily oral administration of tolbutamide on the diabetogenic action of somatotropin.

A purified preparation of bovine somatotropin* was given to male dogs weighing from 10 to 17 kg. The hormone was injected intramuscularly in a daily dosage that varied from 3 to 10 mg./kg. The animals were given a constant daily diet, and any food not eaten by 4:30 P.M. was removed and weighed. Between 8 and 9 every morning, the animals were weighed, venous blood samples were taken, and somatotropin was injected; following these procedures, the dogs were fed. In some instances blood samples for sugar determination were taken at intervals throughout the day. The daily urine excretion was collected quantitatively. The concentration of sugar in the blood and urine was determined by Nelson's method.⁴⁹

One group of somatotropin-treated animals was given a single intravenous injection of tolbutamide† (25 mg./kg.) at various intervals before, during, and after the course of injections of somatotropin. Samples of blood were drawn from the fasted animals at intervals before and for 3 hours after the injection of the tolbutamide. On the day of the test the administration of somatotropin was withheld until the test was completed. A second group of dogs was similarly treated with somatotropin and, in addition, was given a single oral dosage of tolbutamide (50 to 100 mg./kg./day).

The administration of somatotropin (3 to 8 mg./kg./day) for from 4 to 6 days results in a change in the character of the hypoglycemic response to the intravenous injection of a standard dose of tolbutamide. Whereas the normal dog responds to tolbutamide within 30 min. with a precipitous decrease in the blood sugar concentration, the animal treated with somatotropin for 4 to 6 days responds with a relatively slow, progressive decrease. The inhibition of the first phase of the normal acute response to tolbutamide may occur even before the hormone has produced a significant hyperglycemia.⁵⁰

* The somatotropin used in this study is a growth hormone preparation provided by the Endocrinology Study Section, National Institutes of Health.

† We are indebted to The Upjohn Company, Kalamazoo, Mich., for supplies of tolbutamide (Orinase).

Illustrative of the effect of somatotropin on the hypoglycemic response to the injection of tolbutamide are the data depicted in FIGURE 1 which are typical of those obtained in 4 dogs. Prior to the treatment with somatotropin; the injection of tolbutamide resulted in a maximal decrease in the blood sugar within 30 min. Five days after beginning the injection of somatotropin (8 mg./kg./day) the character of the hypoglycemic response to the tolbutamide changed markedly in that the blood sugar decreased

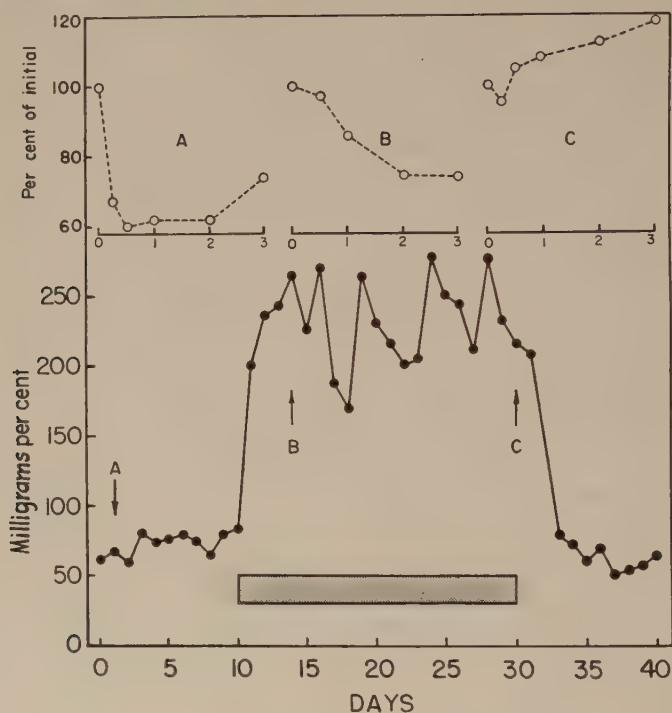


FIGURE 1. Effect of somatotropin on the acute hypoglycemic response to tolbutamide. The dog was given intramuscular injections of somatotropin (8 mg./kg./day) throughout the period designated by the stippled area. An intravenous injection of 25 mg. tolbutamide was given at designated periods (shown by arrows), and the blood sugar (o---o) change is expressed in terms of the initial concentration.

gradually instead of precipitously. On the twenty-third day of somatotropin treatment, the injection of tolbutamide produced a hyperglycemic instead of a hypoglycemic response. Likewise, no hypoglycemic response to the injection of tolbutamide occurred in other dogs that developed metahypophyseal diabetes.

The early effect of somatotropin on the response to the injection of a single dose of tolbutamide can be attributed to a decrease in the stores of available insulin in the pancreas consequent to an increase in peripheral requirements. The gradual decrease in the blood sugar concentration, however, reveals that the pancreas is still capable of secreting insulin. The character of this

response is similar to that which usually follows the administration of a single dose of tolbutamide by mouth to "responsive" patients with diabetes.⁵¹ On the other hand, the hyperglycemic response to the tolbutamide after 22 days of injections with somatotropin is similar to that frequently observed in the completely depancreatized dog. Although the mechanism responsible for the hyperglycemic response is unknown, the data suggest the absence of functioning islet tissue. In some instances this inference was validated by the persistence of the hyperglycemia after the injections of somatotropin

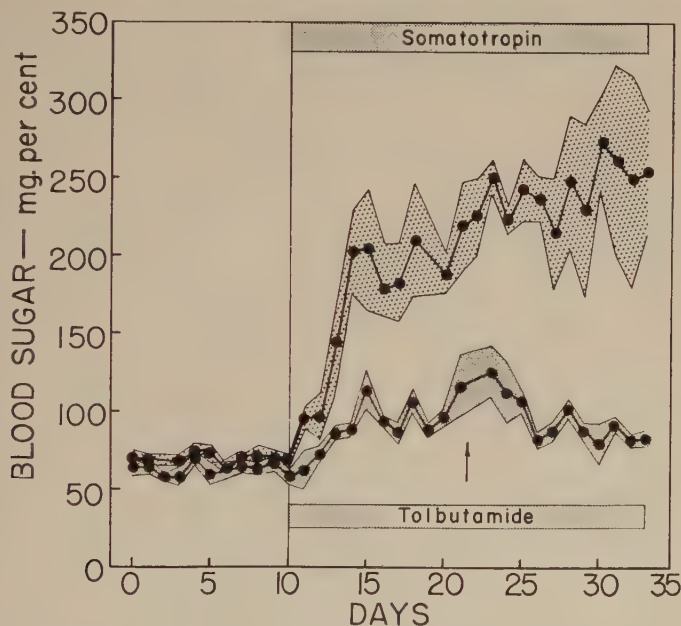


FIGURE 2. Effect of tolbutamide on the hyperglycemic response to somatotropin. The daily fasting blood sugar concentrations (mean \pm S.E.) of 5 dogs injected with somatotropin (6 mg./kg./day) are depicted by the stippled area, and those of 4 dogs injected with the same dosage of somatotropin and fed tolbutamide (50 mg./kg./day) are depicted by the shaded area.

were stopped, that is, by the development of metahypophyseal diabetes. In the experiment illustrated in FIGURE 1, however, the blood sugar returned to normal concentrations, suggesting that the islet tissue was not destroyed completely in spite of the hyperglycemic response to the tolbutamide.

In contrast with the above are the studies designed to evaluate the effect of the frequent oral administration of tolbutamide on the response to somatotropin. The data illustrated in FIGURE 2 represent the response of the blood sugar concentration (mean \pm S.E.) to the administration of somatotropin (6 mg./kg./day) to 5 dogs and of the same daily dosage of somatotropin plus tolbutamide (50 mg./kg.) to 4 dogs. It is apparent that a significant hyperglycemia developed within 5 days after the treatment with somatotropin was started, and that the hyperglycemia persisted throughout

the period of treatment. The response of the dogs that were given both somatotropin and tolbutamide, however, differed from the above in that only a relatively moderate increase in the blood sugar concentration occurred. The difference between the responses of the two groups of animals is statistically highly significant ($p < 0.001$).

Illustrative of the responses of individual animals comprising each group of dogs are the data depicted in FIGURES 3 and 4. By the fifth day dog 34

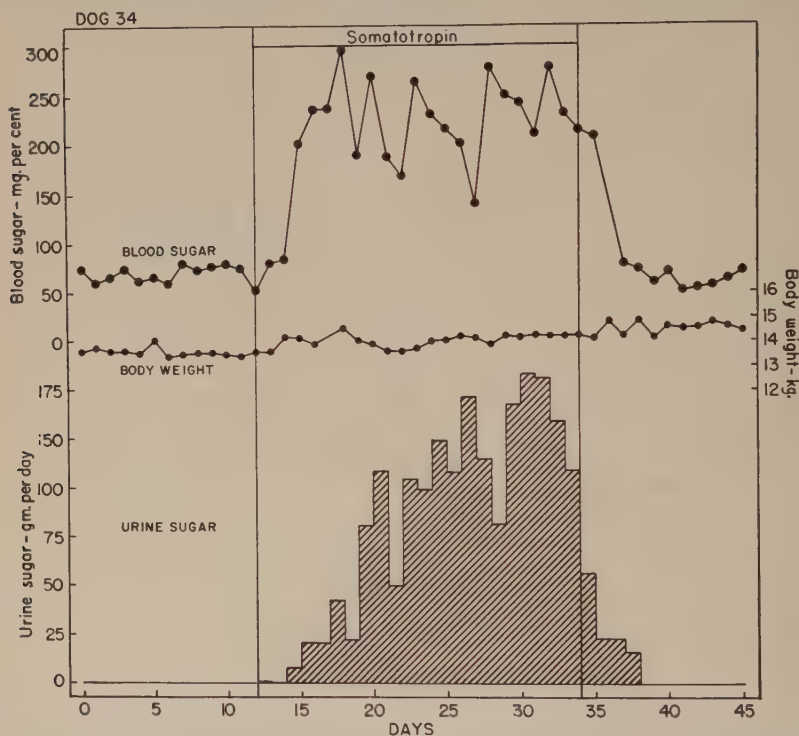


FIGURE 3. The effect of tolbutamide on the diabetogenic action of somatotropin. Dog 34 was given intramuscular injection of somatotropin (6 mg./kg./day) throughout the designated period.

(FIGURE 3) developed a significant hyperglycemia and glycosuria that persisted throughout the period during which somatotropin was injected. Dog 29 (FIGURE 4), however, received tolbutamide and developed a hyperglycemia for only 4 days, and then the blood sugar remained at essentially normal concentrations throughout the period of injections with somatotropin.

Since the individual animals were found to vary markedly in their responsiveness to the diabetogenic action of the somatotropin, experiments were designed in which each animal served as its own control. Six dogs were given 2 courses of injections with somatotropin; 3 received tolbutamide orally during the first course, and 3 were given the tolbutamide during the second course of injections. Illustrative of the first group of dogs are the

data on dog 31, illustrated in FIGURE 5. In this instance the tolbutamide was not started until a definite hyperglycemia and glycosuria were present. Although the blood sugar remained above the normal concentration, it was significantly lower than that produced in the same dog when it was not given any tolbutamide during the second course of injections, which was started after an interval of 13 days.

That the relatively short interval between the 2 courses of somatotropin injections is not responsible for the more marked diabetogenic response to the second course of injections and for the development of metadiabetes in dog

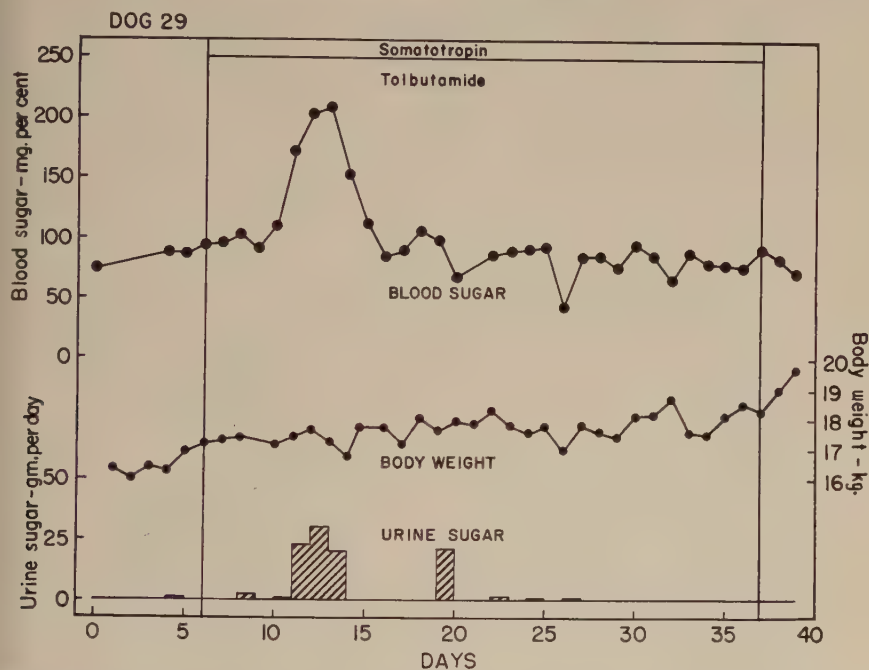


FIGURE 4. The effect of tolbutamide on the diabetogenic action of somatotropin. Dog 29 was given the same dosage of somatotropin (6 mg./kg./day) as dog 34 (see FIGURE 3), plus tolbutamide (50 mg./kg./day orally).

31 (FIGURE 5) is indicated by the data on dog 38 (FIGURE 6). This dog was given intramuscular injections of somatotropin (8 mg./kg./day) and, after a significant hyperglycemia and glycosuria developed, the animal was given tolbutamide (100 mg./kg.) on the days indicated. The blood sugar fluctuated between normal and hyperglycemic levels, and a moderate degree of glycosuria occurred. The second course was started 340 days after the first course of injections of somatotropin was stopped. It is quite apparent that, in spite of the lengthy interval between the 2 courses of injections, a much more marked hyperglycemia and glycosuria occurred during the second course, and the animal developed a metadiabetes.

In complete agreement with the above are the data obtained in the experi-

ments in which the tolbutamide was given during the second course of injections of somatotropin. Typical of these are the data illustrated in FIGURE 7. Whereas a significant hyperglycemia and glycosuria developed during the course of injections of somatotropin, a marked inhibition of the diabetogenic response occurred during the second course, when tolbutamide was given daily by mouth. In no instance did a metahypophyseal diabetes develop after this second course of injections.

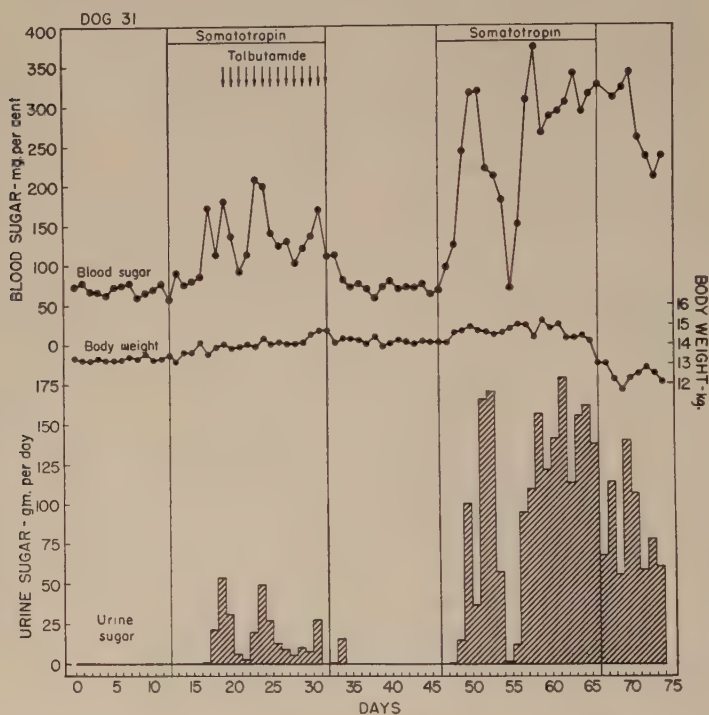


FIGURE 5. Effect of tolbutamide on the diabetogenic action of somatotropin. During the first course of intramuscular injections of somatotropin (5 mg./kg./day), tolbutamide (100 mg./kg.) was given orally at designated days (arrows). A metahypophyseal diabetes followed the cessation of the second course of injections of somatotropin.

Illustrative of another experimental design that was used in this study are the data depicted in FIGURE 8. Five days before beginning the administration of somatotropin (5 mg./kg./day), dog 37 was started on an oral dose of tolbutamide (25 mg./kg./day). The tolbutamide was increased to 50 mg./kg./day on the tenth day after the first injection of somatotropin. During the interval a hyperglycemia developed gradually. Within 2 days after the tolbutamide dosage was increased, the blood sugar concentration began to fall and reached the pretreatment level by the fifth day. On the twenty-sixth day after the first injection of somatotropin (that is, 11 days after the blood sugar concentration reached the control level) the tolbutamide

was discontinued, but the injection of somatotropin (5 mg./kg./day) was continued for another 27 days. The blood sugar remained unchanged throughout this period. When the dosage of somatotropin was increased to 10 mg./day there was no change in the fasting blood sugar concentration even though there was a higher concentration of glucose in the samples taken at 4 P.M. With the injection of somatotropin (15 mg./kg./day) the fasting blood sugar increased and remained elevated even when the dosage was

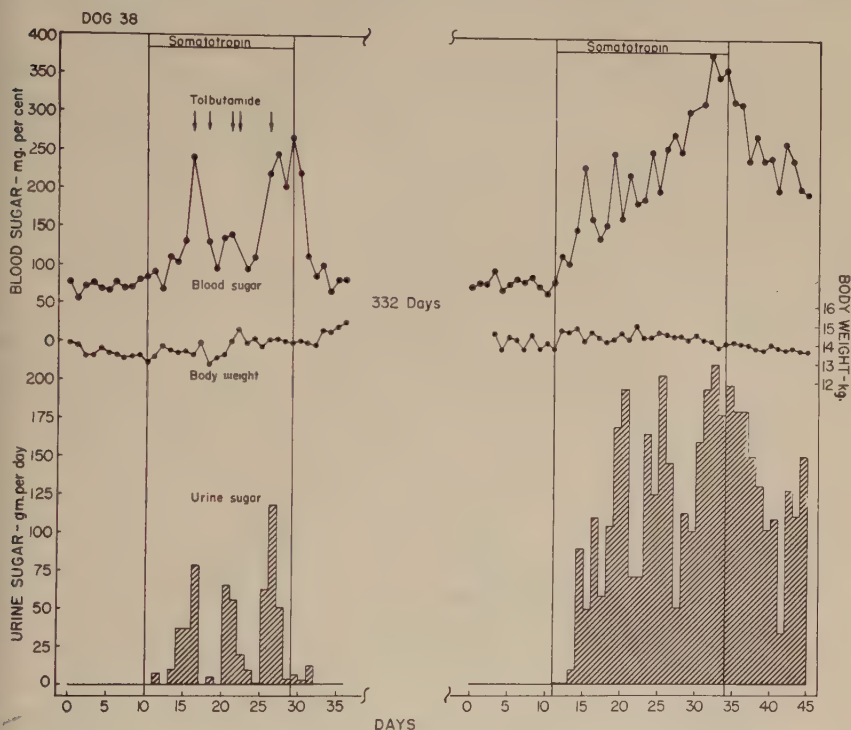


FIGURE 6. Effect of tolbutamide on the diabetogenic action of somatotropin. During the first course of intramuscular injections of somatotropin (8 mg./kg./day), the animal was given tolbutamide (100 mg./kg.) orally at designated days (arrows). A metahypophyseal diabetes followed the cessation of the second course of injections with somatotropin.

reduced to 10 mg./kg./day. With the cessation of somatotropin injections the blood sugar returned to normal levels.

The observations reported herein establish the fact that the administration of appropriate quantities of tolbutamide decreases or inhibits the diabetogenic response to the intramuscular injection of somatotropin. Such an effect could not be anticipated on the basis of the morphologic changes in the pancreas that follow the administration of each agent. As noted above, each agent stimulates the pancreas and induces degranulation and "hydropic degeneration" of the beta cells. If such stimulation and the resultant morphologic changes play a role in the subsequent destruction of the islet

tissue, then the concomitant administration of both agents would be expected to enhance the rate of beta cell destruction and accelerate the development of metadiabetes. The fact that this did not occur raises questions about the validity of the concept that metahypophyseal diabetes is caused by excessive stimulation of the pancreas, with a consequent exhaustion and degeneration of the beta cells.

It can be postulated that the tolbutamide induces a discharge of insulin by direct stimulation of the pancreas and that the resultant increase in circulating hormone nullifies the effects of an increase in peripheral requirements

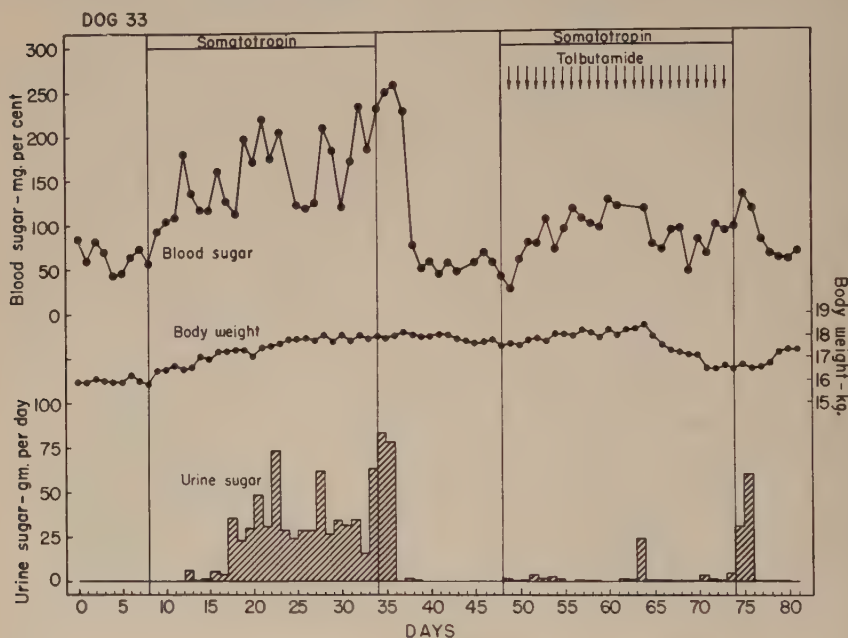


FIGURE 7. Effect of tolbutamide on the diabetogenic action of somatotropin. During the second course of intramuscular injections of somatotropin (5 mg./kg./day) tolbutamide (50 mg./kg./day) was given orally.

for insulin, thereby obviating the diabetogenic action of somatotropin. The observations illustrated in FIGURE 1, however, suggest that tolbutamide is ineffective in producing a further stimulation of the discharge of insulin after the first few days following the onset of somatotropin injections. Further, blood samples taken at various intervals during the day from animals treated with both somatotropin and tolbutamide usually showed that the fasting blood sugar was the lowest of the day.

An alternative hypothesis is suggested by Anderson and Long's⁵² observation that the perfused pancreas stops its secretion of insulin when a growth hormone preparation is added to the perfusate. Thus, somatotropin may exert some direct action on the beta cells that inhibits their secretory activity. Accordingly, the effect of tolbutamide could be attributed to an action in the

beta cell that protects some intracellular mechanism which is vulnerable to the action of somatotropin. It is difficult, however, to postulate a mechanism whereby such a protective action could be effective for as long a period as that observed in dog 37. However, the observation that pretreatment with tolbutamide results in an amelioration of the diabetes that is subsequently produced by the injection of alloxan⁴¹ supports the possibility that tolbutamide exerts some protective action in the beta cells. Further clarification may come with similar and more extensive studies with other diabetogenic agents.

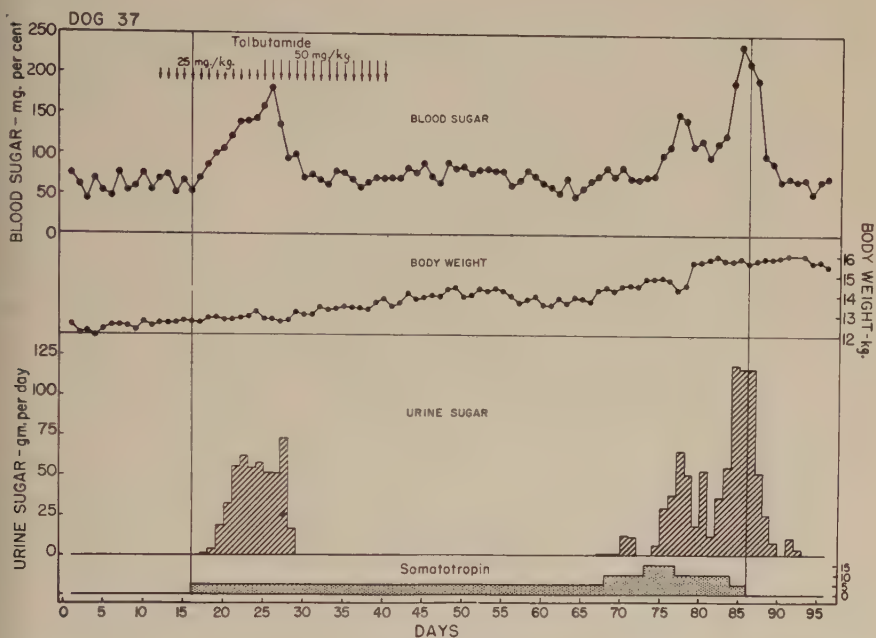


FIGURE 8. Effect of tolbutamide on the diabetogenic action of somatotropin (see text).

The data presented herein offer no new insight to the mechanism responsible for the diabetogenic action of somatotropin. They do suggest that the "growth-onset" type of diabetes mellitus is not due to the action of somatotropin. In contrast with the effect of tolbutamide on the somatotropin-treated dog, the diabetic child who is responsive to tolbutamide at the onset of the syndrome usually loses his responsiveness with the passage of time, irrespective of whether he is treated with tolbutamide⁵³ or with insulin.⁵⁴ The patient with acromegaly, however, who develops diabetes should be responsive to tolbutamide until such time as the beta cells of his pancreas are destroyed. Accordingly, it is probable that an idiohypophyseal diabetes is responsible for the hyperglycemia of patients with acromegaly who have been found to respond to tolbutamide.⁵⁵ The prophylactic action of tolbutamide in such patients must await further study.

Summary

The intravenous injection of a single dose of tolbutamide (25 mg./kg.) to normal dogs results in a precipitous decrease in the blood sugar concentration within 30 min. Within a few days after the beginning of the daily intramuscular injection of somatotropin the injection of a similar dose of tolbutamide results in a gradual decrease in the blood sugar. Subsequently, with the continued administration of somatotropin, the hypoglycemic response to the injection of tolbutamide is completely inhibited.

The diabetogenic effect of the daily intramuscular injections of somatotropin is decreased or inhibited by the concomitant oral administration of tolbutamide.

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DEGRADATION OF INSULIN- I^{131} AND GLUCAGON- I^{131} AND FACTORS INFLUENCING IT*

Robert H. Williams, Janet S. Hay, Margaret B. Tjaden

Department of Medicine, University of Washington, Seattle, Wash.

As recently reported,¹⁻³ insulin is rapidly degraded in the body and apparently before some of the hormone has exerted its action. A better understanding of the factors influencing the degradation of this hormone conceivably could be of assistance in controlling diabetes. In this paper are reported results of some of our studies of the capacity of slices and homogenates of most of the body tissues to degrade insulin- I^{131} . Also are reported the influence on this hormone's degradation of infection, trauma, and many factors that presumably influence the amount of insulin secretion, including starvation, phlorhization, alloxan diabetes, prednisolone, insulin hypophysectomy, adrenalectomy, or diets high in carbohydrate, fat, or protein.

Since glucagon also exerts a powerful influence on carbohydrate distribution and, indeed, may cause diabetes,⁴ certain phases of the metabolism of this hormone are reported.

METHODS

For these studies, insulin and glucagon labeled with I^{131} were used. Since we have reported many previous observations^{1, 5, 6} with these labeled compounds, details of the techniques employed are discussed in this paper only very briefly. However, the following points need particular emphasis. Great caution must be exercised in the iodination of the hormones, because heavy iodination and/or radiation will abolish their chief biological activity and alter their chemical and physical behavior. With the iodination techniques employed in our preparations, full biological activity can be preserved. However, there still tends to be some variation from one batch to the next in some of the chemical properties, necessitating the performance of a sufficient number of control experiments with each batch. For the minimization of the adsorption of the hormones to glassware and for completeness in the precipitation of the hormone it is desirable to have at least several milligrams of protein in the container; we commonly used normal plasma. In order to work with the most satisfactory ratios of enzyme to substrate we routinely added the corresponding nonlabeled hormone.

All insulin- I^{131} used was purchased from the Abbott Laboratories, North Chicago, Ill., and was dialyzed at 4° C. for several hours immediately before use. An insulin-degrading enzyme preparation was used and consisted either of tissue homogenate (liver in most instances) or of the dried powder remaining after special treatment of beef liver homogenate with acetone and ether.

* The work reported in this paper was supported in part by Grants A-575 and A-2456 from the National Institute of Arthritis and Metabolic Diseases, Public Health Service, Bethesda, Md., and in part by the Warren Orr Research Fund, and The Diabetes Teaching and Research Foundation, Seattle, Wash.

The portion of 4 mg. of the extracted liver that was soluble in 0.5 ml. of buffer or a specific amount of tissue homogenate was placed in a beaker. Either 0.067 M phosphate or, more often, 0.1 M tris-(hydroxymethyl)amino-methane (Tris), containing 5×10^{-3} M Versene, was used as a buffer. To the enzyme preparation was added 0.4 μ g. of insulin- I^{131} and 0.1 mg. of amorphous insulin; in this system the quantity of enzyme is the limiting factor in the rate of the reaction. The total 2 ml. mixture, with the pH adjusted to 7.4, was placed in a Dubnoff metabolic shaker at 37° C. for 5 min. Fifteen seconds before the end of the incubation 1 ml. of 2 per cent of plasma in water was added to ensure complete precipitation of insulin; at the end of the incubation period 3 ml. of 20 per cent trichloroacetic acid (TCA) was added. After standing for 30 min., the mixture was centrifuged. The solid residue was washed 2 to 5 times with 10 per cent trichloroacetic acid, and the washings were added to the supernatant. Each sample was run in triplicate.

The radioactivity in each specimen was measured by a well-type gamma counter. The precipitate was dissolved in 30 per cent KOH and brought to a volume equal to that of the combined supernatants. A calculation was then made of the percentage of the total radioactivity found in the supernatant fractions; this fraction was regarded as an indicator of the amount of degraded hormone. At least the following 2 control studies were conducted simultaneously with each experiment: (1) 1 ml. of buffer plus 1 ml. of insulin solution, and (2) 0.5 ml. of buffer plus 0.5 ml. of enzyme preparation plus 1 ml. of insulin solution. The exceptions to the foregoing technique are described where indicated.

All rats were male and, except as noted later, were of the Sprague-Dawley strain.

INSULIN- I^{131} DEGRADATION BY HOMOGENATES OF DIFFERENT TISSUE

Nonfasted rats weighing approximately 200 gm. were exsanguinated, and approximately 300 mg. of tissue was removed from the liver, kidney, and gastrocnemius. Each tissue was added to cold 0.067 M phosphate buffer at pH 7.5 and homogenized. From 1 to 15 per cent concentrations of the homogenates were incubated with insulin- I^{131} (0.5 μ c., 1 μ g.) and 13 μ g. of amorphous insulin. The amount of degradation was determined as described under METHODS.

As seen in TABLE 1, there was an increase in the degradation of insulin- I^{131} , with an increase in the concentration of homogenate used. In concentrations from 1 to 7.5 per cent the effect was essentially linear, in spite of the fact that the experiments were not conducted simultaneously or with the same batch of insulin- I^{131} . Liver and kidney were much more active than gastrocnemius muscle.

Our studies were extended to determine to what extent other tissue homogenates might degrade insulin- I^{131} . The methods used have been described earlier. Red blood cells were isolated and washed thrice with cold phosphate buffer; 0.15 ml. of the cells was used in the assay. In the studies with plasma 0.15 ml. was used in each vessel.

The results are shown in TABLE 2. Since 6 different experiments were con-

TABLE 1
INSULIN-I¹³¹ DEGRADATION BY DIFFERENT CONCENTRATIONS OF
TISSUE HOMOGENATES

Experiment No.*	No. tissue specimens	Concentration of homogenate (per cent)	Average % insulin-I ¹³¹ degraded		
			Liver	Kidney	Muscle
1	4	1	13.1	9.5	6.6
	4	3	32.8	20.5	5.0
2	5	5	32.1	30.1	11.5
3	5	7.5	62.3	51.3	19.7
	5	15	74.4	64.3	25

* The 3 experiments were conducted at intervals separated by weeks, and different batches of insulin-I¹³¹ were used.

ducted, with different batches of insulin-I¹³¹ and with animals of various sizes at widely separated intervals, all of the results are recorded with reference to the diaphragm, to which is assigned a value of 100. It is seen that essentially all of the tissues caused some degradation of the hormone. Among the more active tissues were liver, pancreas, kidney, and testis. On the other hand, plasma had no activity, and washed red blood cells had essentially none. The action of brain, diaphragm, gastrocnemius, and lymph node was relatively small; in the case of muscle, however, its body mass is so great that its total action is very significant.

INSULIN-I¹³¹ DEGRADATION BY SLICES OF DIFFERENT TISSUES

Experiments similar to those described with tissue homogenates were conducted with slices of liver, kidney, kidney cortex, pancreas, spleen, diaphragm, heart, gastrocnemius, stomach, small intestines, fat, testis, and brain. Approximately 200 mg. of each of these tissues was obtained from each of 12 male rats. For each 100 mg. of tissue 1.25 μ g. of insulin-I¹³¹ was added, as well as 10 μ g. of amorphous insulin. Upon completion of the incubation which was conducted as with the homogenates, the medium was poured into 1 ml. of 2 per cent plasma. Ten ml. of 20 per cent TCA was then added; the precipitate was washed twice with 2 ml. of 5 per cent TCA. The tissue was washed 4 times with 2 ml. of saline. The washings were combined. Counts of radioactivity in the supernates and washes were as described earlier.

EFFECT OF INFECTION AND TISSUE DAMAGE ON INSULIN DEGRADATION

In view of the fact that, first, the daily insulin requirements for diabetics are greatly increased with infection and trauma, and second, essentially all

TABLE 2
INSULIN- I^{131} DEGRADATION BY VARIOUS RAT TISSUE HOMOGENATES*

Experiment No.†.....	1			2		3		4		5		6	
Rat No.‡.....	1	2	3	4	5	6	7	8	9	10	11	12	13
Liver.....						464	423	570	657	616	761	249	252
Pancreas.....	251	257	264	278	299	376	328	364	414	859		736	582
Kidney.....										516	540	217	231
Kidney cortex.....	217	235	265	252	261	410	335	362	419	458	619	178	227
Testis.....	291	264	282	291	295	458	412	376	408	325	419	114	149
Small intestines.....	178	299	258	225	216	131	131	191	169	248	322	135	165
Stomach.....	141	188	144	191	199	329	232	204	237	460	825	384	337
Spleen.....	189	169	190	199	205	361	342	248	314	348	543	172	139
Heart.....	139	174	141	109	135	343	309	273	237	159	194	70	96
Diaphragm.....	100	100	100	100	100	100	100	100	100	100	100	100	100
Gastrocnemius muscle.....						143		84	119	75	87	45	45
Brain.....	102	94	101	110	100	222	249	115	108	45		19	12
Adipose.....	54	52	54	27	33	113	96	88		22	30	22	21
Thymus.....	149	145	132	164	178								
Lymph nodes.....	102	81	71	80	92								
Red blood cells.....	5	2	1	4	7								
Plasma.....	0	0	0	0	0								

* All values are related to the action of diaphragm, which was arbitrarily given a value of 100.

† The various experiments were conducted over an interval of 3 years (see text).

‡ In rats 1 to 9, 100 to 150 mg. of tissue was used per beaker; in rats 10 to 13, 15 mg.; in all instances the ratio of tissue weight to insulin weight was 10:1. In the experiments where only 15 mg. of tissue homogenate was used, 100 mg. of bovine plasma albumin was added in order to reduce adsorption of the hormone to glass.

tissues can degrade insulin, we were prompted to ascertain whether increased insulin degradation was demonstrable under these circumstances.

Effect on Degradation of Insulin- I^{131} in Vitro of Serum from Diabetics with Infection

As previously mentioned, normal serum has been shown not to degrade insulin, but it was considered possible for the serum of infected diabetics to act differently. Therefore, through the courtesy of Joseph Crampton, serum was obtained from 2 diabetics, each of whom was suffering from a carbuncle, the development of which caused the need for about twice the usual daily dosage of insulin. Incubation of 1 ml. of serum with insulin- I^{131} for 1 hour, like that of serum of 4 normals studied simultaneously, caused no degradation of the hormone.

Degradation of Insulin- I^{131} in Diabetics and Nondiabetics with Infection

Insulin- I^{131} was injected intravenously as described by Welsh *et al.*,⁷ and plasma samples were obtained 1 hour later from 11 diabetics and 8 nondia-

betics with infection. Approximately one half of each group of subjects had fever at the time of the test; in a few patients the infection was severe (for example, pneumonia). The amount of TCA-precipitable radioactivity in the serum 1 hour after insulin-I¹³¹ administration was compared with large series of noninfected diabetics and nondiabetics.⁷ There was no evidence that the infections were associated with increased degradation. In 2 diabetic patients the test was repeated when the subject was free of infection. In one, the results of the 2 tests were identical; in the other, there was actually less degradation with the infection, because there was more binding to plasma globulin.

In view of the fact that there were many variables that might have influenced the insulin degradation, the problem was studied more specifically in animals and *in vitro*.

Effect of Staphylococci on Insulin-I¹³¹ Degradation in Vitro

Because of the fact that staphylococci are such common offenders in diabetics, a total of 5 *in vitro* experiments was conducted with 2 highly virulent strains of this organism.

Experiment 1. A heavy broth culture of staphylococci was centrifuged, washed twice with saline and then suspended in saline. One half of the material was subjected to supersonic vibration for 45 min. to effect cellular disruption. The material was then recentrifuged, and part of the supernatant was tested as such, whereas another part was boiled before testing. Thus there were 3 preparations of washed staphylococci: (1) whole cells, (2) supernatant from disrupted cells, and (3) a portion of the second preparation that had been boiled. Each preparation was incubated for 5 min. with insulin-I¹³¹ and 4 mg. of amorphous insulin alone or with the insulins plus liver "insulinase." As the results shown in TABLE 3 indicate, none of the preparations of staphylococci caused insulin degradation. Indeed, the boiled staphylococcal supernatant actually decreased the degradation by the insulinase. This may have resulted from increased binding of insulin-I¹³¹ to the boiled staphylococcal products.

TABLE 3
EFFECT OF STAPHYLOCOCCI ON INSULIN-I¹³¹ DEGRADATION

Insulin-I ¹³¹	Liver enzyme	Other	Per cent degradation*
+			1.1
+	+		12.7
+		Washed staph.	1.1
+	+	Washed staph.	13.0
+		Sonified staph.	1.4
+	+	Sonified staph.	11.6
+		Boiled sonified staph.	1.4
+	+	Boiled sonified staph.	7.3

* Average of triplicate determinations.

Experiment 2. In this experiment the incubation was conducted at pH 6.0 and 7.5; only the latter was tested in the previous experiment. In this experiment there was used 80 mg. (dry weight), per incubation flask, of staphylococcal products in the supernatant and 16 mg. of the whole organisms. Again, the staphylococci caused no degradation.

Experiment 3. The staphylococci in this and the next 2 experiments were obtained from one of the aforementioned diabetics with carbuncle and increased insulin requirements. The bacterial material was prepared as previously described. However, the incubations were with only 0.025 as much amorphous insulin and were for 1 hour instead of for 5 min. The determinations were at pH 7.5 and 6.0. Again, no evidence of degradation by the staphylococci was observed.

Experiment 4. The previous experiment was repeated, using a much greater concentration of staphylococci or their products and with one half as much amorphous insulin. No degradation from the bacteria was observed.

Experiment 5. Experiment 4 was repeated except that 10 mg. of bovine plasma albumin was added to each flask in order to minimize adsorption of insulin- I^{131} to glassware and/or products in the medium. There was no significant change in the results.

Effect of Staphylococcal Infection in Rabbits on Insulin- I^{131} Degradation by Muscle

All rabbits used were large white males.

Experiment 1. Tourniquets consisting of a rubber band and a copper wire were placed high on the right thigh of each of 2 rabbits. Seven hours later the tourniquets were removed, and the gastrocnemius as well as surrounding structures were injected with 0.2 cc. of a heavy suspension of staphylococci obtained from a diabetic patient, as described earlier. Five hours thereafter the animals were killed by a blow on the head. About 12 gm. of traumatized and staphylococci-injected muscle was removed, as well as a comparable amount of muscle from the other hind leg. After weighing, this material was homogenized in approximately 40 ml. of 0.1 M Tris in a Waring Blendor. Following centrifugation, the supernatant was removed, and 2 ml. was incubated for 30 min. with 1 ml. of insulin- I^{131} containing 5 μ g. of amorphous insulin. The amount of insulin- I^{131} degraded was determined in the manner described previously. There was no difference in the degradation caused by the muscle extract from the 2 legs.

Experiment 2. An experiment similar to that above was conducted with 5 rabbits, but 1 ml. of a heavier suspension of the staphylococci was injected into each hind leg. The supernatants from the muscle homogenate each contained 22 mg. of solids per 1 ml. Each incubation flask contained 13 μ g. of insulin. There was no difference in the degradation caused by the various extracts.

Effect of Staphylococcal Infection in Rabbits on Insulin- I^{131} Degradation by Serum

In the same white male rabbits described in the previous section, serum specimens were studied. About 15 ml. of blood was withdrawn before the

tourniquets were applied and a few minutes after removing the tourniquets and massaging the leg; in the second experiment an additional blood specimen was withdrawn 20 hours after initiation of the experiment. Two ml. of serum was incubated for 15 min. with 4 or 13 μ g. of insulin in 1 ml. Tris along with a small amount of insulin-I¹³¹. None of the serum specimens caused degradation of the hormone.

Effect of Staphylococcal Infection in Rats on Insulin-I¹³¹ Degradation in Vivo

Under ether anesthesia, each of 6 male rats weighing approximately 250 gm. was injected intraperitoneally with 0.5 ml. of a heavy suspension of staphylococci obtained from the previously discussed diabetic patient. Six days later, following a 24-hour fast, each rat received 30 μ c. of insulin-I¹³¹ in a tail vein. Thirty minutes thereafter, under ether, blood was removed from the aorta in a heparinized syringe. The amount of degraded and undegraded insulin was measured. An average of 49 per cent of the insulin-I¹³¹ was degraded and, in another group of 6 rats treated in the same manner except for the injection of staphylococci, there was a 50 per cent degradation of insulin-I¹³¹.

Effect in Rats of Infection with Fecal Contents on Insulin-I¹³¹ Degradation in Vivo

Five ether-anesthetized rats were injected subcutaneously with 2 cc. of a heavy fecal suspension in water. Eighteen hours later insulin-I¹³¹ was injected into the tail vein, as in the previous experiment. The average degradation was 43 per cent whereas, in 5 controls treated in the same manner except for the injection of fecal suspension, the average degradation was 42 per cent.

Effect of Necrosis of a Rabbit Leg on the Capacity of the Serum or Damaged Muscle to Degrade Insulin-I¹³¹ in Vitro

Experiment 1. A rubber band and a copper wire were wrapped tightly around the upper part of the right hind leg of each of 2 rabbits. Five hours later the tourniquet was removed and the leg massaged for 2 min. Approximately 15 ml. of blood was withdrawn from the heart before the tourniquets were applied, as well as 2 min. and 20 min. after their removal. To 1 ml. of serum was added 4 mg. of amorphous insulin, 10 μ g. insulin-I¹³¹, and 2 ml. of Tris buffer. The mixture was incubated for 1 hour, and the amount of insulin-I¹³¹ degraded was measured. None of the serum specimens promoted degradation.

Experiment 2. Under ether anesthesia the right thighs of 7 rabbits were severely crushed with a hammer. This procedure subjected the animals to severe stress, as evidenced by the fact that many died. The serum from blood drawn before the crushing, 2.5 hours and 8 hours later, when incubated for 15 min. with insulin-I¹³¹ caused no degradation of the latter. About 10 gm. of muscle was removed from each thigh and homogenized in saline. One aliquot of the supernatant from a 25 per cent muscle homogenate was adjusted to pH 7.5 with Tris buffer, and another to pH 6.0 with phos-

phate. One ml. of each of these mixtures was incubated for 15 min. with 30 μ g. insulin and insulin- I^{131} in 1 ml. of water. All tests were conducted in duplicate. The amount of insulin- I^{131} degraded by the traumatized and nontraumatized muscle extracts was comparable. Experiments with the control muscle were conducted, using twice or 0.5 the concentration of the muscle extract used above. The degradation of insulin- I^{131} was proportionate to the amount of extract.

Effect of the Crushing of a Rat Leg upon the Degradation of Insulin- I^{131} in Vivo

Under ether anesthesia the right hind legs of 6 male rats were severely crushed with a hammer. The process was repeated 4 days later, and the animals were then fasted for 24 hours. Insulin- I^{131} was injected into a tail vein and, after 30 min., blood was removed from the aorta and analyzed for degraded and undegraded hormone as previously described. Six other rats were handled exactly in the same manner, excluding the trauma. The average amount of degraded insulin- I^{131} in the controls was 50 per cent and in the traumatized group 55 per cent ($p < 0.001$). Three months later the experiment was repeated with 6 rats in similar manner, except that only one traumatization was inflicted, 24 hours before sacrifice, and the rats were not fasted. The amount of degradation was 47 per cent in the traumatized group and 41 per cent in the controls ($p < 0.01$).

EFFECT ON INSULIN- I^{131} DEGRADATION OF CONDITIONS PRESUMABLY
INFLUENCING INSULIN SECRETION

Many chemical agents in the body in some manner exert a contra-insulin action. Since insulinase markedly degrades insulin, we were prompted to investigate in rats the effect on the insulinase action of many individual factors, listed in TABLE 4, that influence carbohydrate metabolism and, presumably, the secretion of insulin. In each individual experiment there were 6 rats, except for one alloxanized group and the adrenalectomized group, each of which included 4 rats. Thus, there were 116 experimental rats with completed studies and 60 controls. The high-fat diet consisted of a ratio of 1 gm. of peanut oil to 1 gm. of ground Purina Fox Chow.* The high-carbohydrate diet consisted of 3 parts of fox chow to 7 parts sucrose, and the high-protein diet contained 7 parts casein per 3 parts of fox chow; thus, each of the 3 diets contained more than 75 per cent of its calories as fat, carbohydrate, or protein. Twelve gm./rat/day was served, and all was devoured. The phlorhizinized and alloxanized rats showed persistent glycosuria. Ultralente insulin was injected subcutaneously once daily. For the experiments with inactive insulin, an ultralente preparation was treated with cysteine. Prednisolone acetate was injected subcutaneously once daily. As shown in TABLE 4, some rats were of the Sprague-Dawley strain and some were Wistars, some were fasted and others not; some were treated on the day of sacrifice and others not. All rats were weighed at the beginning of the experiment (approximately 250 gm.) and on the day of sacrifice. All of the procedures were rigorous, especially the prednisolone and insulin

* Product of Purina Mills, Buffalo, N. Y.

TABLE 4
EFFECT ON INSULIN-I¹³¹ DEGRADATION BY LIVER HOMOGENATES OF CONDITIONS PRESUMABLY INFLUENCING INSULIN SECRETION

Treatment			Rats				Insulin-I ¹³¹ degraded					
Type	Days	Daily dose/rat	On day of expt.	Fasted	Sprague-Dawley	Wistar	%/15 Mg. wet weight	%/15 Mg. dry weight	%/Mg. protein	Mg./whole liver	Mg./100 gm. body weight	
							Expt.* Con.	Expt. Con.	Expt. Con.	Expt. Con.	Expt. Con.	
Starvation.....	4				+	+	5.5 5.3	17.6 16.4	1.5 1.6	{1.8 4.0}	{0.9 1.3}	
High-fat diet.....	6		0	+	+	+	5.9 5.3					
High-fat diet.....	6		+	0	+	+	6.5 5.3	{20.1 16.4}†	2.1 1.6	3.7 4.0	1.4 1.3	
High-protein diet.....	22		0	0	+	+	5.0 5.3					
High-protein diet.....	10		0	0	+	+	5.3 5.3	17 16.4	1.5 1.6	4.0 4.0	1.4 1.3	
High-CHO diet.....	22		0	0	+	+	6.5 5.3					
High-CHO diet.....	10		+	0	+	+	5.4 5.3	16.9 16.4	1.9 1.6	3.7 4.0	1.3 1.3	
Phlorhizin.....	13	0.1 gm./kg. q.d.	0	+	+	+	3.7 3.8					
Alloxan.....	35			0	+	+	4.1 4.4	10.0 10.7	1.3 1.4	1.9 3.5	{1.6 0.9}	
Alloxan.....	13		0	0	+	+	5.6 4.2	{17.6 13.4}	1.6 1.3	3.1 2.9	1.5 1.1	
Adrenalectomy.....	13		0	0	+	+	4.4 4.4	10.0 10.7	1.5 1.4	3.2 3.5	{1.5 0.9}	
Hypophysectomy.....	13		0	0	+	+	4.8 4.4	10.0 10.7	1.3 1.4	3.0 3.5	0.9 0.9	
Ultralente insulin.....	7	6 U.	0	0	+	+	2.3 2.3	7.7 6.6	0.7 0.7	1.6 1.9	0.6 0.5	
Ultralente insulin.....	6	6 U.	+	0	+	+	{5.1 3.8}	16.8 12.2	1.3 1.2	3.1 2.7	1.1 1.0	
Ultralente insulin.....	6	6 U.	+	0	+	+	2.3 1.8			1.6 1.3	0.5 0.4	
Ultralente insulin (inactive).....	13	8 U.	0	0	+	+	4.6 3.9			3.2 2.8	1.1 0.9	
Ultralente insulin (inactive).....	6	60 U.	+	0	+	+	4.9 3.8	16.1 12.2	1.5 1.2	3.3 2.7	1.2 1.0	
Prednisolone acetate.....	8	5 mg.	0	+	+	+	{3.0 4.5}					
Prednisolone acetate.....	10	5 mg.	0	0	+	+	3.7 3.8	11.5 12.2	1.1 1.2	2.3 2.7	1.2 1.0	
Prednisolone acetate.....	8	5 mg.	+	0	+	+	2.7 3.1			2.5 2.4	{1.5 0.9}	
Prednisolone acetate.....	8	5 mg.	+	0	+	+	1.4 1.8			1.3 1.3	0.5 0.4	

* Expt. = experimental animals; Con. = controls.

† The braced figures represent statistically significant differences ($p < 0.01$).

administrations. A number of rats in these groups died. The alloxanized, starved, and prednisolone-treated rats lost a great deal of weight. Those on high-protein or high-fat diets and those phlorhizinized lost a small amount; the adrenalectomized, hypophysectomized, and high-carbohydrate group did not gain. Each rat was decapitated and the liver rapidly removed, washed in 0.1 M Tris buffer, blotted on moist filter paper, and weighed. The liver was homogenized in Tris. A 1 ml. aliquot containing 15 mg. liver (wet weight) was incubated at 37° C. with 90 μ g. of insulin, as well as insulin- I^{131} in 1 ml. Tris for 5 min. Fifteen seconds before the end of incubation, 1 ml. of 2 per cent plasma was added. The reaction was stopped by the addition of 3 ml. of 20 per cent TCA. The mixture was stirred, allowed to stand for at least 30 min., then centrifuged, decanted, and the residues washed twice with 5 per cent TCA. The washes were combined and the radioactivity of the supernatants as well as the precipitates measured, as described earlier. Since different intervals elapse during the preparation of the liver homogenates, studies were first conducted at 4° C. to determine such effects on insulinase activity. Fifteen rats, each weighing about 300 gm., were decapitated, and at 0, 2, 4, 6, and 8 hours thereafter the livers from 3 animals were removed and the insulinase determined; no significant difference was found.

Compared with the controls, which were of comparable body weight at the beginning of the experiments, the total liver weights were significantly less in the following groups: adrenalectomy, hypophysectomy, alloxan, high-fat, and starved; they were increased in the prednisolone and high protein groups. The percentage of water was decreased in the adrenalectomy, hypophysectomy, and the prednisolone groups.

The averages of the other major observations are shown in TABLE 4. Considering the marked changes in metabolism, there were surprisingly few alterations in liver insulinase activity. There was an increase in the degradation of the liver homogenate of one high-fat and one alloxan group when the calculations were based on a liver dry-weight ratio, but not by the other standards. On a liver wet-weight basis there was one insulin group that had an increased degradation and one prednisolone group that had a decreased degradation. On the basis of the total body weight there was an increase in degradation by the adrenalectomized group and one of the alloxan and prednisolone groups. Each of these groups failed to gain weight, and each, except the prednisolone group, had a significantly subnormal liver weight. Considering the radical metabolic changes that were found to be induced by the various procedures, the relatively few abnormalities in liver insulinase were surprising.

GLUCAGON- I^{131} DEGRADATION

Using glucagon labeled with I^{131} in the manner described previously,^{8, 9} it was found that homogenates of pancreas, liver, kidney cortex, and muscle broke down this labeled hormone. Indeed, in concentrations of homogenates from 0.05 to 0.4 per cent and 5 μ g. of glucagon- I^{131} the relations were essentially linear (FIGURE 1). Studies with muscle and liver, however, showed

that the effect tended to plateau when more than 1 per cent concentration was used.

Glucagon-I¹³¹ degradation by acetone precipitates from liver homogenate is inhibited by many of the same compounds that inhibit the insulin-I¹³¹ degradation.^{10, 11} However, there was a different degree of inhibition in some instances. For example, the following inhibited insulin-I¹³¹ degradation more than glucagon-I¹³¹: benzoyl-L-tyrosine ethyl ester, acetyl-L-tryptophan ethyl ester, tannic acid, dithiouracil, bis-(dimethylcarbamyl)

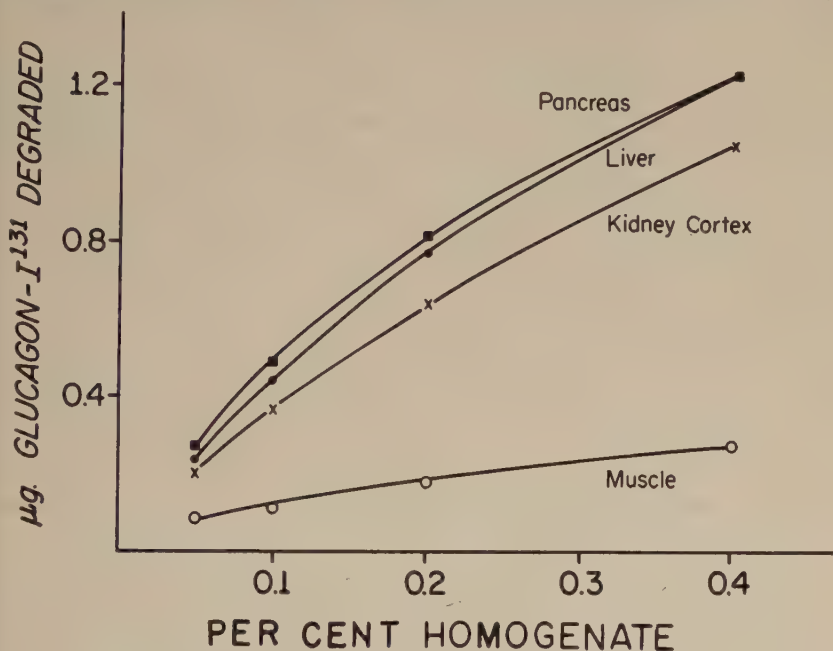


FIGURE 1. The degradation of glucagon-I¹³¹ by varying concentrations of tissue homogenates.

disulfide, and 1-butyl-3-*o*-aminophenylsulfonylurea. A comparable degree of inhibition of degradation of the 2 hormones was observed with phenethylbiguanide and 1-phenyl-3-phenylsulfonylurea.

DISCUSSION

Before discussing our results, consideration will be given to some of the biochemical factors involved in insulin and glucagon degradation. It has long been known that glutathione (GSH) and other such reducing substances inactivate insulin;¹² indeed, the hormone is inactive when only one third of the disulfide linkages have been reduced.^{13, 14} Insulin is inactivated and has its disulfide linkages reduced by a dialyzable heat-stable factor from rat muscle.¹⁵ Insulin-I¹³¹ is degraded by a heat-labile factor from liver¹⁶ and, to some extent, by a boiled liver extract.¹⁷ The dialyzable heat-stable factor could be glutathione, since it definitely degrades insulin-I¹³¹; however,

reduction is not a prerequisite for insulin- I^{131} degradation because an acetone precipitate of liver homogenate degrades insulin- I^{131} even after dialysis for 48 hours.¹⁷ Such a preparation causes much less degradation than an undialyzed one or a dialyzed one to which glutathione has been added;¹⁸ under certain conditions glutathione activity synergizes enzymatic degradation and, conversely, enzymatic action stimulates degradation by glutathione.¹⁸ Rat liver has been shown to contain an enzyme designated as glutathione reductase¹⁹ that catalyzes the reduction of oxidized glutathione (GSSG). This TPN-linked GSSG reductase system does not act directly on insulin, but promotes regeneration of GSH that has been oxidized by insulin; it increases the effectiveness of a given amount of glutathione. There is evidence suggesting that there is a second enzyme in the liver that directly reduces insulin, probably by promoting transfer of hydrogen from GSH to

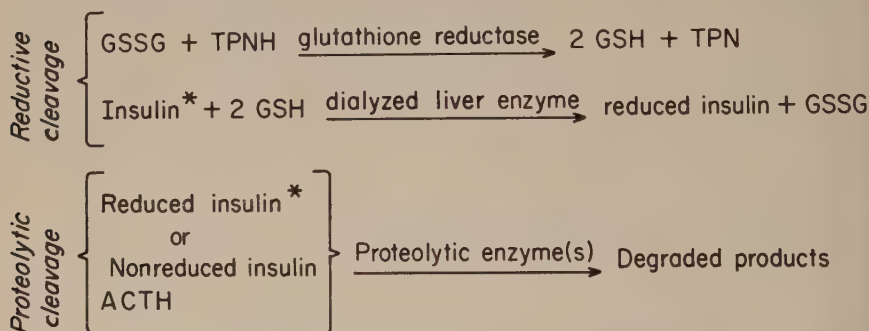


FIGURE 2. Liver enzymes that may be involved in the degradation of insulin and other hormones. Somatotropin, prolactin, vasopressin, or oxytocin also may engage in the reactions indicated by the asterisks.

insulin.¹⁸ These observations are in keeping with a preliminary report by Racker²⁰ suggesting that enzymatic reduction of insulin by liver might occur. In conclusion, as diagrammed in FIGURE 2, it appears that reduction of an enzymatic and nonenzymatic type causes the inactivation and degradation of insulin. However, this presumably is not the only method of degradation, because many observations have suggested¹⁻³ that the liver contains one or more enzyme systems that cause proteolytic degradation of insulin. The use of insulin- I^{131} has been of great assistance in these studies.^{5, 6, 21, 22} Mirsky has presented the hypothesis that there is a single enzyme in liver that has a high degree of specificity for the degradation of insulin.²³ This may prove to be the case, but it has not been possible to establish it as a fact because of the crudity of the enzyme preparations that have always been used;²⁴ such preparations probably contain several enzymes and act on a number of substrates other than insulin. Tomizawa and Halsey²⁵ have recently isolated a protein fraction from beef liver, apparently pure by starch-block electrophoretic and ultracentrifugal patterns, which degrades insulin- I^{131} . However, glucagon- I^{131} degradation is not demonstrated by the usual method. The specific mechanism of degradation of insulin is under investigation.

In the studies conducted with crude liver extract it has been shown that the degradation of insulin-I¹³¹ is decreased by casein, α -corticotropin, glucagon, somatotropin, and certain other proteins.²⁴ On the other hand, other proteins, for instance, albumin,^{22, 24} exert no effect. Actually, many protein hydrolyzates and amino acids, as well as a vast number of other compounds, inhibit insulin-I¹³¹ degradation,^{2, 10, 11, 26, 27} some of the inhibitors are competitive, and some are noncompetitive. The same crude liver enzyme preparation that degrades insulin-I¹³¹ has been shown to degrade glucagon-I¹³¹ also.^{8, 28, 29} The degradation of each hormone is also inhibited by α -corticotropin, α -casein, and somatotropin, nonlabeled glucagon or insulin,^{8, 28} phenethylbiguanide, 1-butyl-3-*o*-aminophenylsulfonylurea, 1 phenyl-3-phenylsulfonylurea, bis-(dimethylcarbamyl) disulfide, dithiouracil, tannic acid, acetyl-L-tryptophan ethyl ester, benzoyl-L-tyrosine ethyl ester, *p*-chloromercuribenzoic acid, and many other compounds, including hydrolyzates of the aforementioned proteins.²³ The fact that the enzyme action is blocked by *p*-chloromercuribenzoic acid and other agents that react with sulfhydryl groups suggests that these groups are important in the enzymatic action. The greatest enzymatic degradation of glucagon-I¹³¹, like that for insulin-I¹³¹, is found in the residual sucrose supernatant of a rat liver homogenate subjected to differential centrifugation.^{8, 29} Each labeled hormone is broken down by most tissues of the body,^{1, 9} the liver and kidney being particularly active.

In spite of the many similarities of enzymatic degradative actions on insulin and glucagon, a number of differences have been demonstrated. For example, GSH greatly increases the amount of degradation of insulin-I¹³¹ caused by a dialyzed liver enzyme preparation, whereas it has little effect on glucagon-I¹³¹ degradation. Iodoacetate and cupric ion, agents that react with glutathione, inhibit insulin degradation more than glucagon.^{30, 31} Aqueous or bicarbonate extracts of rat liver lose their insulin hydrolyzing action on standing overnight in the cold, on dilution, or on blending for 2 min.; these procedures do not have much effect on the amount of glucagon degradation.³² Citrate increases insulin degradation, but does not affect that of glucagon.^{23, 32} One liver preparation has been reported³³ to inactivate glucagon, but not insulin. Conversely, as mentioned earlier, the purified enzyme isolated by Tomizawa and Halsey degrades insulin-I¹³¹ but not glucagon-I¹³¹.

In analyzing the studies reported thus far it appears (FIGURE 2) that in insulin degradation enzymatic and nonenzymatic reductions are important, whereas these reduction processes are not involved in glucagon degradation since glucagon does not contain disulfide bonds. However, proteolysis seems to occur in the degradation of each hormone.^{30, 31, 34} We do not know how and to what extent the reductive and the proteolytic reactions are related. It is possible that other disulfide-containing hormones such as somatotropin, prolactic, oxytocin, and vasopressin may, like insulin, be degraded by both reductive and proteolytic reactions, whereas the degradation of corticotropin, like that of glucagon, may involve proteolysis only.

As previously noted, most tissues have the capacity to degrade glucagon-I¹³¹

as well as insulin- I^{131} ; in each instance the liver, kidney, pancreas, and testis are among the most active. Not much is known about the degradative systems of tissues other than the liver. Muscle contains a nondialyzable, heat-labile system.³⁵ Corticotropin, somatotropin-oxidized insulin, α -casein, and glucagon inhibit the degradation of insulin- I^{131} by muscle and enhance the glucose uptake by rat diaphragm;³⁵ the enhancing effect of glucagon on insulin-increased glucose uptake had been noted earlier by Randle.³⁶ Crude extract of beef anterior pituitary also contains an enzyme system that causes insulin- I^{131} degradation.³⁷ It has been shown that insulin- I^{131} degradation *in vivo* is reduced in hypophysectomized rats.³⁸

In studies with insulin- I^{131} it was shown that tissue slices were much less active than homogenates, although the relative order of effectiveness of the different tissues was similar; this apparently is due to the liberation of the large quantities of enzyme(s) from the soluble intracellular fraction.

Once insulin is bound to protein, it is less subject to degradation.^{7, 39} Indeed, the binding of insulin- I^{131} to plasma globulin accounts for the abnormally slow degradation of insulin- I^{131} in subjects who have had insulin treatment for several weeks or longer;^{7, 40} diabetics not treated with insulin apparently have a normal rate of insulin degradation. Even those with infection were found to have a normal rate of degradation of insulin- I^{131} *in vivo*, and sera from such patients incubated with insulin- I^{131} did not degrade insulin- I^{131} . Many studies were conducted to investigate further whether the increase in dosage of insulin required by diabetics with infection or trauma was due to increased insulin degradation. However, there was no degradation produced by sera of patients or animals infected with staphylococci and no increased degradation *in vivo* with staphylococcal infection or, in rats, infection caused by the subcutaneous injection of fecal suspensions. Whole or fragmented staphylococci incubated with insulin- I^{131} did not degrade it. Severe traumatization of the hind leg of rabbits did not cause the presence of insulin- I^{131} -degrading material in the serum, whether or not there also was infection of the muscle. With muscle traumatization in rats there was demonstrated an increase in insulin- I^{131} degradation *in vivo*.

Because of the fact that insulin- I^{131} is degraded very rapidly in the body and because the liver has a large capacity for this action and has the first chances to react with endogenous insulin, the quantity of insulinase activity in liver was studied in rats subjected to one of several vigorous procedures thought to influence significantly the amount of insulin secreted. These included starvation, phlorhization, alloxan diabetes, injections of prednisolone or insulin, hypophysectomy, adrenalectomy, and diets high in carbohydrate, fat, or protein. Starvation was previously reported to decrease liver insulinase activity,⁴¹ we found that it decreased the total liver insulin- I^{131} -degrading capacity. A few abnormalities were produced by other factors, but we were impressed by the fact that they were relatively small considering the strenuous nature of the procedures. These observations raise the question as to how extensively the insulinase system is involved in the homeostasis for carbohydrate metabolism. However, it always has a potent insulin degrading capacity, and there is evidence that,

the greater the insulin content in the body,^{1, 2} the larger is the total amount degraded.

SUMMARY AND CONCLUSIONS

Essentially all tissues possess the capacity to degrade insulin-I¹³¹ and glucagon-I¹³¹ rapidly; liver, pancreas, kidney, and testis are among the most active. Much more information must be obtained before the mechanism(s) of degradation are established but, in the case of liver, present information suggests that insulin is degraded by enzymatic and nonenzymatic reduction, as well as by proteolysis, whereas glucagon degradation is affected by the latter process alone. Tissue homogenates degrade insulin much faster than tissue slices. Many different compounds inhibit the degradation of insulin and glucagon, some by competitive and others by noncompetitive reactions. Staphylococcal infections in patients, rats, and rabbits do not cause the serum to degrade insulin-I¹³¹. Indeed, neither whole staphylococcal cells nor disrupted cells cause degradation of the hormone. Traumatization of the rabbit leg does not cause the serum to degrade insulin, nor does it increase the degradative capacity of muscle. However, traumatization of rat leg causes an increased degradation of insulin-I¹³¹ *in vivo*. Of many procedures assumed to influence significantly the amount of insulin secreted, few change the degradative capacity of liver to a biologically significant degree. The conditions tested were: starvation, insulin or prednisolone injections, hypophysectomy, adrenalectomy, alloxan diabetes, phlorhization, and diets high in carbohydrate, fat, or protein. Additional study is needed to determine the role of insulin-degrading enzymes in homeostasis and in the pathogenesis of diabetes.

ACKNOWLEDGMENTS

We thank Henry H. Tomizawa and H. T. Narahara for helpful advice concerning the techniques involved in studying insulin degradation and for stimulating discussions concerning the possible mechanisms by which insulin, as well as other protein and peptide hormones, are degraded. We are also grateful to Elaine Henley for assistance in some of the studies with patients.

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DETERMINATION OF THE RATE OF INSULIN DESTRUCTION IN VIVO*

Thaddeus E. Prout and Ieuan E. Evans

Department of Medicine, The Johns Hopkins University School of Medicine, Baltimore, Md.

Insulin given by intravenous injection disappears rapidly from the plasma. The half time of insulin labeled with iodine-131 (I^{131}) in the intravascular space has been shown to be approximately twenty-five minutes.¹ The rapid decline in plasma concentration is the result of several events that occur simultaneously: first, insulin undergoes proteolytic destruction in the liver and kidney;² second, insulin may be bound or sequestered by tissue cells;³ and, third, insulin may be dispersed in the compartments of body water. The experiments reported in this paper were designed to determine the extent to which each of these processes affects insulin disappearance.

Methods

Normal male rabbits were used in this study. Animals were sacrificed at the end of each experiment by intravenous injection of lethal quantities of pentobarbital. Complete urine collections were obtained by excision of the bladder. The experiment was discontinued if spontaneous voiding occurred. Eviscerated animals were prepared by removal of the intestinal tract from the lower esophagus to the rectum, including the spleen and pancreas, under barbitol narcosis and ether anesthesia. The arterial blood supply to the liver was ligated and sectioned. Barbitol narcosis was continued in these animals throughout the experiment. Nephrectomy was performed either alone or in association with evisceration, as described in the experimental detail.

Isotonic sterile saline was added to radioinsulin† to form a concentration of approximately 5 μ c./ml. The solution was stored at 10° C.

At the start of each experiment each animal was given intravenous injection of 2 ml. of a 10 per cent solution of NaI. A 20-min. period of circulation of the NaI was allowed in order to block uptake of free I^{131} by the thyroid gland. Crystalline zinc insulin was then given by intravenous injection to some of the animals. Radioactive insulin was administered intravenously 10 min. after the injection of crystalline zinc insulin. A 50 per cent solution of glucose (1 to 2 ml. I.V.) was used to prevent hypoglycemia.

The volume of injected radioinsulin was measured accurately in a syringe calibrated by means of a threaded brass rod; the plunger of the syringe was advanced by a screw. In order to determine the quantities of insulin injected, an aliquot of the material that had been drawn into the syringe was

* The work reported in this paper was supported in part by a grant from the American Cancer Society, New York, N. Y., and by Research Grant A-2041 and Graduate Training Grant 2A-5136 from the National Institute of Arthritis and Metabolic Diseases, Public Health Service, Bethesda, Md.

† Insulin- I^{131} was obtained from Abbott Laboratories, Oak Ridge, Tenn.

added to 50 ml. of pooled human plasma (serum phantom). Analysis of the radioactivity in this serum phantom gave an accurate measure of the characteristics and quantity of radioactive material injected into the rabbit.

The analysis of both the serum phantom and of the rabbit serum were carried out by the same technique on 0.1 ml. of serum. Intact insulin was isolated by electrophoresis or hydrodynamic flow.^{1, 4} The precipitable radioactivity was obtained by adding 1 ml. of 10 per cent trichloroacetic acid (TCA) to 0.1 ml. of serum. The precipitate was separated by centrifugation and the radioactive supernatant fluid was poured off. Determination of the quantity of total radioactivity was obtained by adding that contained in the TCA precipitate to that in the TCA-soluble fractions. The quantity of total circulating radioactivity was also checked by placing 0.1 ml. of serum on Whatman No. 3 filter paper as in the procedure for electrophoresis. However, in this instance the paper was placed immediately in 3 cc. KOH. Radioactivity in all samples was counted in a shielded well-type scintillation crystal connected through a linear preamplifier to a scaler. The gamma emissions of the I^{131} were measured to eliminate errors of interference. All counts were carried out to sufficient numbers to reduce the counting error to less than 2 per cent.

TABLE 1
PERCENTAGES OF DEVIATION FROM THE MEAN OF THREE CONSECUTIVE SERUM ANALYSES

Compartments	Deviation from mean			Average
Total radioactivity.....	+1	-3	+1	±1.4
TCA precipitate.....	+1	0	-2	±1.0
TCA soluble.....	+1	-3	+1	±1.4
Insulin.....	-5	+2	+1	±2.6
Average error, per cent total count.....				±1.6

Determinations of the radioactivity in the TCA-precipitable and the TCA-soluble insulin and of the total compartments were carried out in duplicate on both the serum of the rabbit and the serum phantom. Three consecutive serum phantoms were compared for accuracy. The deviations of determinations of each of the serum compartments from their mean are shown in TABLE 1. The average error introduced by each analysis was 1.6 per cent of the total counts.

Results

It has been demonstrated that insulin labeled with I^{131} can be separated from radioactive proteins in serum more accurately by electrophoresis than by precipitation with trichloroacetic acid. The assumption that all radioactivity precipitated from the serum by trichloroacetic acid is biologically active insulin was not supported by previous work from this laboratory.¹ The importance of using a technique with which the compartments can be

clearly distinguished is shown in FIGURE 1. The first column shows the characteristics of the radioactive material assayed in serum immediately after its arrival from the manufacturer. Ninety-two per cent of the radioactivity in solution can be precipitated by trichloroacetic acid. The remaining 8 per cent is in the TCA-soluble fraction. Only 86 per cent of the total radioactivity can be isolated as insulin by electrophoresis. Subtraction of the quantity of radioactivity isolated as insulin by electrophoresis from the TCA-precipitable fraction leaves the radioactive fraction due to biologically inactive material. Columns 2 and 3 show the characteristics of the material at 4 and 8 days, respectively. The insulin fraction is now 77 and 74 per cent of total radioactivity; the inactive fraction has risen from 4 to 15 and 17

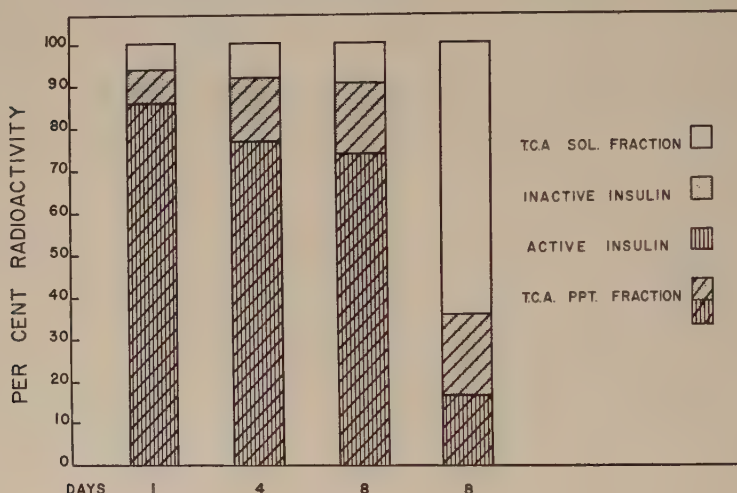


FIGURE 1. Characteristics of the radioactive solution. Radioactive solution stored at 10° C. was added to serum on the days shown. Analysis was carried out immediately following mixing. Composition of the radioactivity in solution changes gradually with time. The fourth column shows the change in the characteristics of the solution kept at room temperature over an 8-day period.

per cent, respectively, over this period. The fourth column shows the characteristics of a part of the material that was separated from the original, but left at room temperature. The insulin content is reduced to 17 per cent of the total radioactivity after 8 days; the TCA-soluble fraction is 64 per cent of the total radioactivity. It is important to note that the characteristics of the radioactive solution may change completely. Unless the characteristics of the radioactive solution are followed, reduction in the insulin content as shown here will be undetected. This will alter the results obtained and studies done at different times will not be comparable.

The biologically inactive material migrates with the serum protein on electrophoresis of the serum. There is no detectable degradation of this fraction over a period of several hours and it continues to recirculate in the plasma.¹ Therefore, the TCA-soluble radioactivity that appears in the

plasma or serum following injection of the radioactive solution comes from the degradation of the insulin. These products of degradation will hereafter be designated as soluble I^{131} .

The rate of insulin destruction *in vivo* may be determined by the following technique. The amount of radioactivity injected as insulin- I^{131} and the soluble I^{131} are measured by analyses of the serum phantom. At the end of the experimental period blood is drawn for determination of the serum concentration of insulin and soluble I^{131} . The bladder is removed from the intact animals for determination of soluble I^{131} that has been excreted in the urine. The soluble I^{131} resulting from insulin degradation that is not

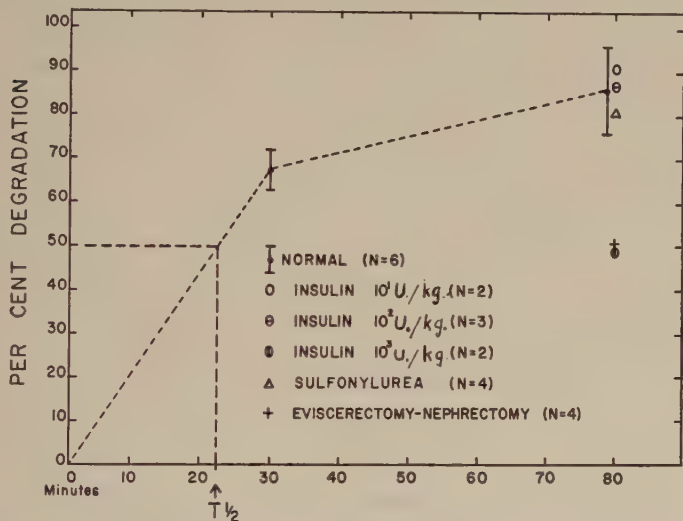


FIGURE 2. Insulin degradation in the rabbit. Degradation of radioinsulin injected in the rabbit has been studied under various conditions. Prior administration of insulin in dosages of 10^1 and 10^2 U./kg. did not alter the degree of degradation. Sulfonylurea (tolbutamide, chlorpropamide) had no effect on degradation. Insulin in dosage of 10^3 U./kg. in the intact animal decreased degradation to the level of that in the eviscerated-nephrectomized animal.

excreted is calculated by multiplying the I^{131} content of the serum by the iodine space. The iodine space was found to be 26 per cent of body weight and is in agreement with previous determinations.⁵ The intact insulin remaining at the end of the experiment is the difference between the injected radioactivity and the total degradation products.

The per cent of insulin degradation at 30 min. and 80 min. in the normal rabbit is shown in FIGURE 2. At 30 min. after injection an average of 68 per cent of injected radioinsulin has undergone separation of the radioactive label. This is assumed to be the result of proteolytic destruction of the insulin molecule. The half time of insulin destruction is 22 min. This correlates closely with the half time of the disappearance of radioactivity from the plasma, as previously reported.¹ At 80 min. an average of 86

per cent of the injected radioinsulin has been degraded. This may be contrasted to the rate of degradation of insulin in the eviscerated-nephrectomized rabbit. As shown in FIGURE 2, the degradation of insulin was decreased to 51 per cent at 80 min. under these circumstances.

In order to slow the process of degradation in the normal rabbit, an attempt was made to saturate the degrading mechanisms with crystalline zinc insulin. Dosages of crystalline zinc insulin of 1, 10, 100, and 1000 U./kg. body weight were injected 10 min. prior to the injection of radioinsulin. The rate of insulin degradation was not influenced by quantities of insulin up to 100 U./kg. body weight. When 1000 U. of insulin/kg. body weight were injected prior to the radioinsulin, the rate of degradation was reduced to 48 per cent at

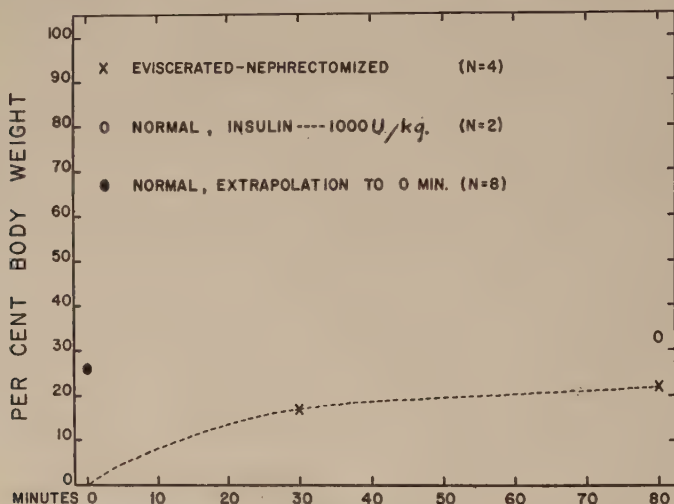


FIGURE 3. The apparent volume of distribution of insulin in the rabbit is equivalent to the volume of the extracellular space in the insulin-treated (10^3 U./kg.) and eviscerated-nephrectomized rabbit. Previous determinations in the normal animal¹ are also shown for comparison.

80 min. It is clear that the degrading mechanisms for insulin are difficult to saturate and far exceed their physiological need.

The possibility that the sulfonylurea compounds act by decreasing insulin destruction is suggested by some of the studies done *in vitro* with enzyme systems.⁶ On the other hand, no change in the rate of disappearance of radioinsulin following tolbutamide administration was noted.⁷ The effect of tolbutamide and chlorpropamide on insulin degradation has been studied with the present technique. As shown in FIGURE 2, there was no decrease in the rate of insulin destruction following the use of these drugs in the normal rabbit.

In order to clarify the role of tissue binding in the disappearance of insulin, the space of distribution of insulin must also be determined. It is probable that tissue binding and the space of distribution are closely related. A determination of insulin space was made in earlier studies from this

laboratory in which it was assumed that the rate of degradation was constant from the time of injection. Following injection of radioinsulin, determinations of the insulin content of serum were made between 30 and 80 min. after injection. The rate of insulin disappearance was extrapolated to zero time. The insulin content of serum at zero time was divided into the quantity of insulin given by injection. By this calculation the insulin space was found to be approximately 26 per cent of total body weight.¹ The insulin space was not altered by prior administration of sulfonylurea.⁷

The present technique has been applied also to the study of the insulin space. The quantity of intact insulin remaining at 30 or 80 min. divided by the insulin concentration per ml. of serum at the same intervals gives the apparent volume of distribution of the intact insulin. Insulin destruction by the liver and kidney was eliminated in these experiments in order to clarify the effect of the peripheral tissue on insulin disappearance. As shown in FIGURE 3, the insulin space was found to be 16 per cent of body weight at 30 min. and 22 per cent of body weight at 80 min. in the eviscerated-nephrectomized rabbit. Also shown are the results obtained when degradation is decreased by the prior intravenous administration of insulin in a dosage of 1000 U./kg. The results in FIGURE 3 are in accordance with the previous estimations of the insulin space^{1, 8} and are approximately the size of the extracellular space.

The implications of this apparent volume of distribution of insulin should be considered further. The quantity of insulin necessary to saturate the binding sites for insulin in muscle has been estimated by Stadie⁹ to be approximately 1.5 μ g. of insulin/gm. of tissue. Under the conditions of the experiments reported here, the availability of tissue binding sites must greatly exceed the available insulin. If intact insulin were captured by peripheral cells without degradation, the quantity of insulin remaining in the periphery would increase, while the concentration of circulating insulin would become progressively smaller. Therefore, the apparent volume of distribution of the intact insulin would become progressively larger and finally become indeterminate. The apparent volume of distribution found in these experiments is inconsistent with this assumption. The radioactive insulin injected can be accounted for by the summation of the insulin subjected to proteolytic destruction and the insulin contained in extracellular water. This leads to the interpretation that the degradation of intact insulin is not prevented by tissue binding or sequestration. The similarity between the space occupied by intact insulin and the extracellular compartment suggests, rather, that insulin is degraded quickly after leaving the extracellular space. The clinical implication of these results is that intact insulin cannot be saved for future use by peripheral tissues.

Summary

Factors influencing the rate of disappearance of insulin from plasma have been studied by the use of insulin labeled with iodine-131 (I^{131}). A technique has been employed that measures the amount of active intact insulin administered. Some change in the characteristics of the radioactive solution on

standing for short periods makes this necessary. In the intact animal an average of 86 per cent of the injected insulin was degraded in 80 min. The rate of degradation was not influenced by prior administration of sulfonyl-urea compounds.

The apparent volume of distribution of insulin *in vivo* is equivalent to the volume of extracellular water. Sequestration of intact insulin without degradation is not a factor in the rapid disappearance of insulin from plasma. The radioactive insulin injected can be accounted for by the summation of the insulin subjected to proteolytic destruction and the insulin contained in extracellular water. Under the conditions of these experiments, there is no evidence that the degradation of intact insulin is prevented by tissue binding or sequestration.

Acknowledgments

We thank Lorraine Conway and Beverly Smith for their technical assistance.

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A COMPARISON OF THE INFLUENCE OF TOLBUTAMIDE AND SMALL DOSES OF INSULIN ON THE SPLANCHNIC OUTPUT AND PERIPHERAL UPTAKE OF GLUCOSE IN MAN*

James W. Craig, William R. Drucker,† Max Miller, Hiram Woodward, Jr.,
Valerie Molzahn

The Departments of Medicine and Surgery, The School of Medicine, Western Reserve University, The University Hospitals of Cleveland and Highland View, The Cuyahoga County Hospital, Cleveland, Ohio

Considerable evidence has been offered to support the hypothesis that the administration of a sulfonylurea to a human subject or animal with an intact pancreas is followed by the release of a substance with hypoglycemic activity, presumably insulin, from the pancreatic beta cells.¹ The refractoriness of fully alloxanized animals and the influence of the beta cell reserve on the character of the early hypoglycemic response to the drugs² are compatible with this hypothesis. In addition, pancreatic vein blood from dogs given a sulfonylurea reduced the blood glucose concentration of both normal³ and alloxan-diabetic⁴ recipient dogs in cross-circulation experiments and produced hypoglycemia in mice.⁵

The unqualified acceptance of insulin as the mediator of sulfonylurea-induced hypoglycemia has been tempered by the failure of many investigators to demonstrate an insulinlike effect of the drugs on the peripheral tissues.⁶ In addition, the sulfonylureas have been shown to reduce the rate of glucose release from the liver,⁷ while the existence of a direct effect of insulin on hepatic glucose metabolism has been questioned.⁸

Although an increase in the peripheral arteriovenous (A-V) blood glucose concentration difference has usually been observed in human subjects following the intravenous administration of a single dose of 0.1 U. insulin/kg., or a total of 5 to 7 U.,^{9, 10} doses of 3 U. or less have frequently failed to produce a demonstrable increase in this measure of peripheral glucose metabolism.^{11, 12} From the results of studies with C¹⁴-labeled glucose in diabetic and non-diabetic subjects, Jacobs *et al.*¹³ concluded that the intravenous administration of 6 to 20 U. of insulin augmented the rate of glucose removal from the body glucose pool and suppressed the introduction of newly released glucose into the pool (glucogenesis), presumably from the liver. When insulin was given by the subcutaneous route in order to retard its rate of entrance into the circulating blood, 10 U. decreased the rate of glucogenesis without affecting glucose removal. A reduced net splanchnic output of glucose (NSGO) has been observed in man after either tolbutamide^{12, 14} or insulin.¹⁵ These findings of various investigators suggested that in man small doses of insulin

* The work reported in this paper was supported in part by the Diabetes Research Program of Western Reserve University, through Research Grant A-1317 from the National Institute of Arthritis and Metabolic Diseases, Public Health Service, Bethesda, Md., and the Fund for the Study of Diabetes Mellitus and Related Metabolic Diseases at Western Reserve University.

† Markle Scholar in The Medical Sciences.

might have the same effects as the sulfonylureas on the NSGO and the peripheral uptake of glucose.

This possibility has been tested by comparing the effects of tolbutamide and small doses of insulin on the NSGO and the simultaneously measured peripheral A-V blood glucose concentration difference in man.

Methods

The subjects for these studies were 25 nondiabetic hospital patients who were tested after an overnight fast. Eight of the subjects were tested with sodium tolbutamide,* 11 received glucagon-free crystalline insulin;† the other 6 served as control subjects and were given only 0.9 per cent sodium chloride solution. These substances were administered into a peripheral vein during a 2-min. interval in the following doses: 2 gm. of sodium tolbutamide, either 2 or 3 U. of insulin, and 50 ml. of sodium chloride solution. Blood samples were obtained simultaneously through indwelling needles in a radial artery and an antecubital vein and through a radiopaque catheter with its tip in a hepatic vein. Simultaneous arterial, peripheral venous, and hepatic venous blood samples were obtained at 5- or 10-min. intervals during a 15- to 35-min. preliminary control period and for 50 min. after injection of the test substance. The glucose content of the blood samples was determined in duplicate by the titrimetric method of Somogyi;¹⁶ by this method a total of 23 determinations on 4 blood samples yielded a mean value of 85 mg./100 ml. with a mean standard deviation of 2.1 mg./100 ml. The hepatic blood flow was estimated by the Bromsulphalein technique of Bradley *et al.*,¹⁷ with measurement of the plasma Bromsulphalein concentration by the method of Gaebler.¹⁸ NSGO was calculated as the product of the hepatic venous-arterial (HV-A) blood glucose concentration difference and the estimated hepatic blood flow; a positive value would thus signify a net output of glucose from the splanchnic system and a negative value would indicate a net uptake. The statistical significance of the differences between mean values was assessed by the "t" test of Fischer.

Results

As seen in FIGURE 1, tolbutamide produced an average maximum arterial blood glucose fall of 27 mg./100 ml. in 40 min. that was still maintained at 50 min. Insulin produced a more rapid fall of similar magnitude, with an average maximum decline of 25 mg./100 ml. in 25 min. and a prompt return toward the fasting level. In the control subjects, who were given sodium chloride, no significant alteration in arterial blood glucose concentration was observed.

The average values for the HV-A blood glucose concentration difference are illustrated in FIGURE 2 and are summarized in TABLE 1. After tolbutamide administration the HV-A difference decreased in all 8 subjects. In 7 of the 8 subjects the average HV-A difference during the first 30 min. after tolbutamide was at least 4 mg./100 ml. lower than the average preliminary control

* Supplied by C. J. O'Donovan of The Upjohn Company, Kalamazoo, Mich.

† Supplied by W. R. Kirtley of Eli Lilly and Company, Indianapolis, Ind.

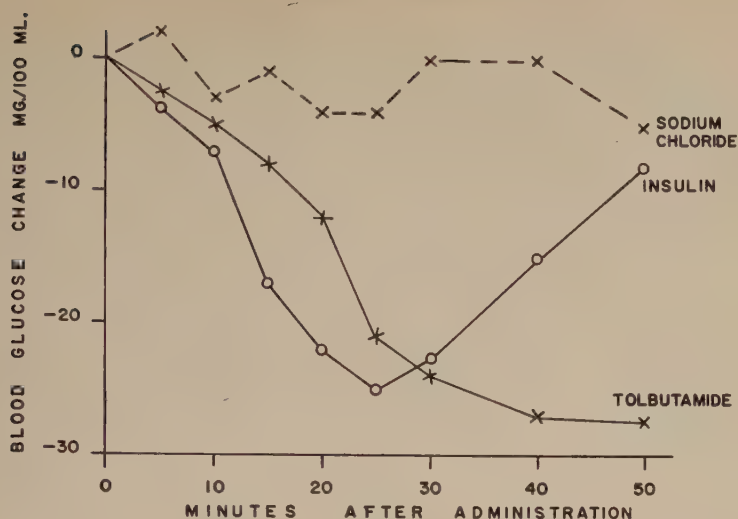


FIGURE 1. Average changes in arterial blood glucose concentration produced by the peripheral intravenous administration of sodium tolbutamide (8 subjects), insulin (9 subjects), or sodium chloride (6 subjects).

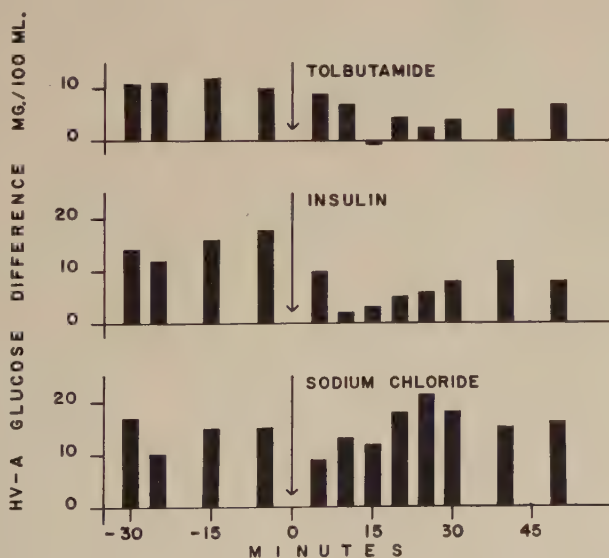


FIGURE 2. Average HV-A blood glucose concentration differences before and after the peripheral intravenous administration of sodium tolbutamide (8 subjects), insulin (9 subjects), or sodium chloride (6 subjects). The first 4 bars on the left in each section represent values obtained during a preliminary control period.

TABLE 1

AVERAGE VALUES (± 1 STANDARD DEVIATION) OF HV-A BLOOD GLUCOSE DIFFERENCE, NSGO, PERIPHERAL A-V BLOOD GLUCOSE DIFFERENCE, AND (A-V)/A RATIO IN NONDIABETIC SUBJECTS BEFORE AND FOR THE FIRST 30 MIN. AFTER THE PERIPHERAL INTRAVENOUS ADMINISTRATION OF SODIUM TOLBUTAMIDE, INSULIN, OR SODIUM CHLORIDE

Substance administered	No. of subjects	HV-A (mg./100 ml.)		
		Before	After	p
Sodium tolbutamide.....	8	10.7 \pm 6.5	4.1 \pm 3.2	<0.05
Insulin.....	9	14.9 \pm 7.6	5.7 \pm 3.0	<0.01
Sodium chloride.....	6	14.1 \pm 5.8	15.3 \pm 10.4	>0.5
		NSGO (mg./min.)		
		Before	After	p
Sodium tolbutamide.....	8	138 \pm 59	50 \pm 29	<0.01
Insulin.....	9	197 \pm 101	73 \pm 25	<0.01
Sodium chloride.....	6	176 \pm 69	199 \pm 120	>0.5
		A-V (mg./100 ml.)		
		Before	After	p
Sodium tolbutamide.....	6	2.2 \pm 2.5	-1.0 \pm 2.4	0.05
Insulin.....	9	2.9 \pm 4.7	1.4 \pm 3.8	>0.4
Sodium chloride.....	4	2.0 \pm 1.7	3.0 \pm 1.9	>0.4
		(A-V)/A ratio		
		Before	After	p
Sodium tolbutamide.....	6	.030 \pm .046	-.021 \pm .036	>0.05
Insulin.....	9	.028 \pm .044	.019 \pm .050	>0.5
Sodium chloride.....	4	.023 \pm .023	.032 \pm .025	>0.5

value. Similarly, after insulin treatment there was a diminution in the HV-A difference in 9 of the 11 subjects. In 8 of these 9 subjects the average decrease exceeded 4 mg./100 ml.* After sodium chloride none of the control subjects showed a decline in the average HV-A difference of as much as 4 mg./100 ml., and there was no significant change in this difference for the control group as a whole.

The average values for the NSGO are illustrated in FIGURE 3 and are summarized in TABLE 1. Tolbutamide decreased the NSGO in all 8 subjects. The average value during the first 30 min. after tolbutamide was at least 40 mg./min. less than the preliminary control value in all 8 subjects. NSGO

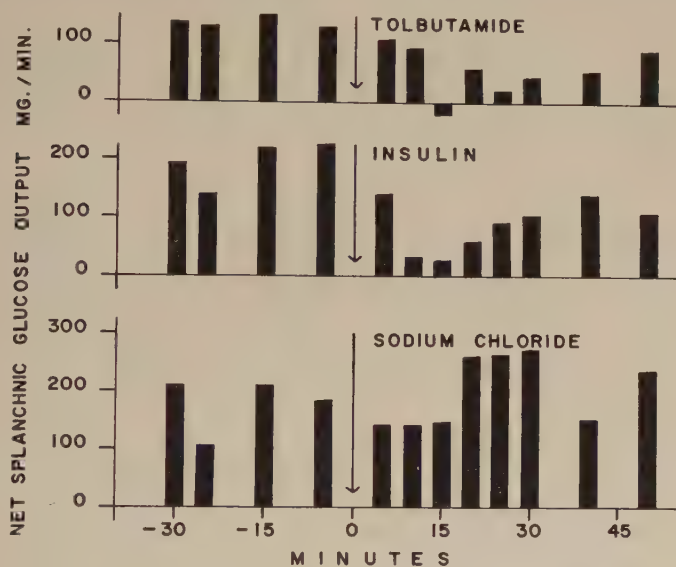


FIGURE 3. Average values for the NSGO before and after the peripheral intravenous administration of sodium tolbutamide (8 subjects), insulin (9 subjects), or sodium chloride (6 subjects). The first 4 bars on the left in each section represent values obtained during a preliminary control period.

decreased after insulin in 9 of the 11 subjects tested; in 8 of the 9 the decrease in the average NSGO exceeded 40 mg./min. After sodium chloride only one of 6 control subjects exhibited a decline in the average NSGO that was greater than 40 mg./min. There was no significant decrease in the average NSGO for the group of 6 control subjects.

Although there were fluctuations in the estimated hepatic blood flow of individual subjects, none of the substances administered produced a significant change in the average flow.

The average values for the peripheral A-V blood glucose concentration difference are illustrated in FIGURE 4 and are summarized in TABLE 1. No apparent increase in the A-V difference was observed during the first 30 min.

* Data from the two subjects in whom there was no demonstrable decrease in the HV-A difference after insulin administration are not included in the figures or table.

after tolbutamide in 5 of the 6 subjects tested. In 3 of these 5 cases the average A-V difference decreased more than 4 mg./100 ml. and in 4 of them the values became negative. During the first 30 min. after insulin there was no increase in the average A-V difference in 6 of the 9 subjects; the increase exceeded 1 mg./100 ml. in only one of the other 3 cases. The changes in the average A-V difference during the first 30 min. after insulin were not statistically significant for the group of 9 subjects. The return of the arterial blood glucose toward the initial fasting level at 40 and 50 min. after insulin administration was accompanied by an increased peripheral A-V difference that was

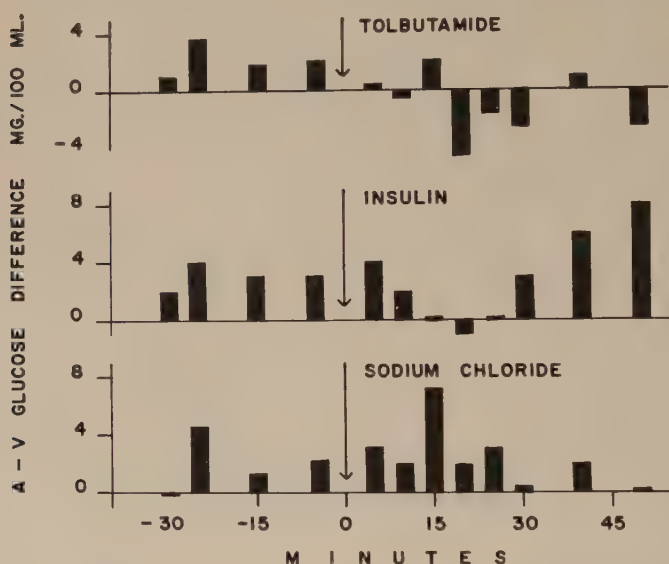


FIGURE 4. Average A-V blood glucose concentration differences in the forearm before and after the peripheral intravenous administration of sodium tolbutamide (6 subjects), insulin (9 subjects), or sodium chloride (4 subjects). The first 4 bars on the left in each section represent values obtained during a preliminary control period.

at least 4 mg./100 ml. greater than the preliminary control value in 5 of the 9 subjects. None of the 4 control subjects tested with sodium chloride showed an increase in A-V difference as great as 4 mg./100 ml. and there was no significant change in the A-V difference for the group as a whole. Because of the influence of the arterial blood glucose concentration on the magnitude of the peripheral glucose uptake,¹⁹ (A-V)/A ratios were calculated; the average values are included in TABLE 1. Changes in the (A-V)/A ratio paralleled the changes in A-V difference.

In summary, both tolbutamide and small doses of insulin produced in nondiabetic human subjects a decline in arterial blood glucose concentration that was accompanied by a decrease in NSGO without an increase in peripheral A-V blood glucose concentration difference demonstrable by the techniques employed.

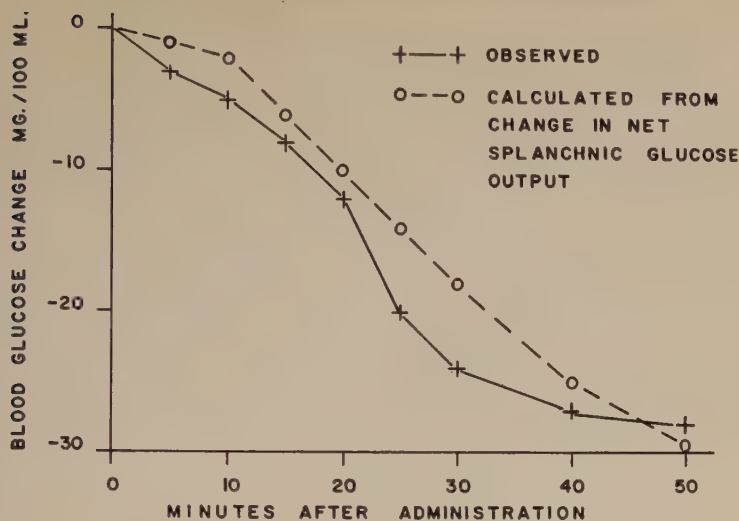


FIGURE 5. A comparison of the average arterial blood glucose concentration changes after tolbutamide administration with the calculated changes that would be produced by the observed alterations in NSGO. The assumptions involved in this calculation are discussed in the text.

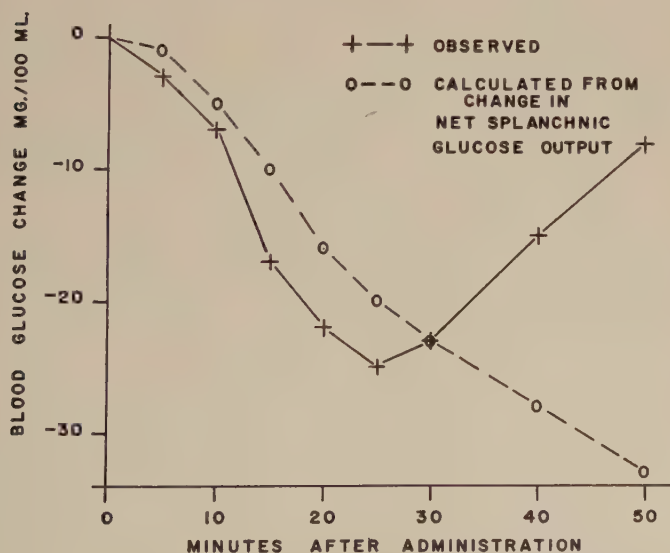


FIGURE 6. A comparison of the average arterial blood glucose concentration changes after insulin administration with the calculated changes that would be produced by the observed alterations in NSGO. The assumptions involved in this calculation are discussed in the text.

Discussion

The techniques employed in this study impose certain limitations on the conclusions that may be drawn from the results obtained:

(1) Since the A-V glucose differences were measured only across the splanchnic bed and in an upper extremity, changes in glucose assimilation in other organs would not be detected.

(2) A decrease in NSGO could result from a diminution in the net hepatic glucose output, an increase in the glucose uptake in an extrahepatic splanchnic component such as adipose tissue, or both. The present results do not allow a distinction among these possibilities and do not permit a decision as to whether tolbutamide and insulin act at the same or different sites within the splanchnic system. Studies with C¹⁴-labeled glucose in animals^{7, 20, 21} and man^{13, 22} have shown that sulfonylurea-induced hypoglycemia is accompanied by an inhibition of glucogenesis, presumably from the liver. From similar studies with insulin, some investigators^{13, 20, 22-25} have concluded that one action of the hormone was a reduction of the rate of glucogenesis, while others^{7, 21} have been unable to demonstrate such an effect consistently. Direct measurements of the hepatic venous-portal venous glucose difference in the dog have shown a decrease in net hepatic glucose output after tolbutamide, but not after insulin administration.⁷ Such direct measurements are not available in man.

(3) The limitations of the analytical method for glucose and the necessity for assuming the constancy of the peripheral blood flow make the measurement of the A-V glucose difference a relatively insensitive technique for the detection of small changes in peripheral glucose uptake. The failure to detect an increment in A-V glucose difference by this technique does not exclude the occurrence of small increases in this difference and, therefore, should not be considered to prove that neither tolbutamide nor insulin in this dosage exerts any peripheral effect. On the other hand, if changes as large as those observed in the splanchnic system occurred in skeletal muscle and skin, there is no reason to believe that they would escape detection since the total blood flow to resting skeletal muscle and skin in man is similar in magnitude to the splanchnic blood flow.^{26, 27}

(4) The present studies do not indicate the mechanism of action of tolbutamide and insulin. The two hypoglycemic agents might have different and independent mechanisms of action even though their sites of action were the same.

(5) In man the sites of action of endogenous and exogenous insulin might differ. Such a discrepancy might be due to differences in their loci of entry into the blood stream,²⁸ alterations produced in the exogenous hormone during its preparation,²⁹ or the heterologous nature of exogenous insulin from animal sources.³⁰

It is of interest to determine whether the observed decrease in NSGO is adequate to account for the fall in the arterial blood glucose concentration that followed tolbutamide or insulin administration. If it is assumed that the rate of glucose uptake by the nonsplanchnic tissues remained constant

and that glucose was distributed uniformly in the extracellular fluid volume, estimated as 20 per cent of the body weight,³¹ the decline in the blood glucose concentration that would result from the observed decrease in NSGO can be calculated. The results of these calculations are shown in FIGURES 5 and 6. Although there are large discrepancies between the predicted and observed blood glucose decreases in individual cases, the average values are similar in magnitude after both tolbutamide and insulin. It is concluded that the decrease in NSGO could be the major cause of the blood glucose fall after tolbutamide or insulin, but the occurrence of an increased glucose uptake in a nonsplanchnic tissue cannot be excluded. In these studies the return of the blood glucose concentration toward the initial fasting level after insulin-induced hypoglycemia is not adequately accounted for by the observed changes in NSGO or peripheral A-V glucose difference.

The similarity of the results obtained with tolbutamide and small doses of insulin appears compatible with an insulin-mediated hypoglycemic action of the sulfonylureas. However, because of the limitations of the techniques employed, other mechanisms of action are not excluded.

Summary

In nondiabetic human subjects the arterial blood glucose decline produced by the peripheral intravenous administration of tolbutamide was accompanied by a reduction in NSGO sufficient to account for the major portion of the blood glucose fall. No increase in the peripheral A-V glucose difference was demonstrated.

Similar changes were produced by small doses of insulin.

The similarity of the effects produced by tolbutamide and insulin is compatible with an insulin-mediated hypoglycemic action of the sulfonylureas. This conclusion must be tempered by recognition of the limitations of the experimental techniques employed.

Acknowledgments

We express our appreciation to T. W. Moir, A. B. Ford, and W. B. Newberry, Jr., who performed the hepatic vein catheterizations, and to Nancy Hofmann, Bryant Kingsbury, and Frank Mills, who gave valuable technical assistance.

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THE RELATIONSHIP BETWEEN THE MECHANISM OF ACTION OF THE SULFONYLUREAS AND THE SECRETION OF INSULIN INTO THE PORTAL CIRCULATION*

Leonard L. Madison, Burton Combes,† Roger H. Unger, Norman Kaplan
*Department of Internal Medicine, The University of Texas Southwestern Medical School,
Dallas, Texas.*

The conclusion that the hypoglycemic effect of the sulfonylureas is mediated through the secretion of endogenous insulin is based upon data indicating that the presence of functional pancreatic beta cells is prerequisite for their hypoglycemic action. In all experimental preparations in which the beta cells are ablated, including completely alloxanized, depancreatized, or eviscerated animals, the sulfonylureas do not significantly lower the blood sugar.¹⁻⁴ By contrast, hypoglycemia eventuates whenever the beta cells are intact,⁵ even in hepatectomized animals.^{6, 7} Recently Pfeiffer has reported an increase in insulinlike activity of the portal venous blood after sulfonylurea administration.⁸

Despite the formidable evidence linking the hypoglycemic effect with the presence of beta cells, objections to the insulinogenic mechanism of action have been raised. For the most part these objections are based upon the failure of the sulfonylureas to duplicate precisely the metabolic effects of exogenous insulin on a variety of parameters. The sulfonylureas are said to produce hypoglycemia by decreasing the hepatic output of glucose.⁹⁻¹² This hepatic effect and the failure of the sulfonylureas to increase peripheral glucose utilization consistently^{13, 14} have been cited as physiological actions inconsistent with the beta-cytotropic mechanism of action. It should be pointed out that such objections implicitly assume that endogenous insulin reduces blood glucose concentration primarily by increasing peripheral glucose utilization and has little or no effect on the hepatic output of glucose.

To conclude from these conflicting data that the sulfonylureas do not stimulate endogenous insulin secretion leaves their dependence on intact beta cells obscure and unexplained. In view of this beta-cell dependence, an alternative conclusion would be that the sulfonylureas stimulate the secretion of endogenous insulin which, in turn, may differ in its physiological effects from exogenous insulin. The natural hormone and its commercial counterpart, exogenous insulin, may differ for several reasons: (1) exogenous insulin may be altered in its commercial preparation; (2) exogenous insulin prepared from one species and administered to another species may not have physiological effects identical to the species-specific endogenous hormone; (3) the metabolic effect of exogenous insulin may be altered because it enters the body through the systemic circulation. Under physiological conditions endogenous insulin is secreted into branches of the portal circulation; there-

* The work described in this paper was supported in part by grants from The Upjohn Company, Kalamazoo, Mich., and The Dallas Heart Association, Dallas, Texas.

† Established Investigator, American Heart Association, Inc., New York, N. Y.

fore, *all* of the natural hormone must traverse the liver before entry into the systemic circulation, whence it becomes available to the peripheral tissues.

The delivery of endogenous insulin to the liver in amounts and concentration greater than those possible for any other tissue in the body suggests that the secretion into the portal circulation has physiological significance. Nevertheless, the controversy as to whether insulin has a direct effect on the hepatic output of glucose still persists.^{11, 15, 16} *In vitro* effects of insulin on the liver have been difficult to demonstrate, but *in vivo* effects of varying duration and magnitude have been reported.^{17, 18} Recently new evidence, both *in vitro*¹⁹ and *in vivo*,²⁰⁻²³ supporting a direct effect of insulin on hepatic glucose metabolism has appeared. The data of Wall,²⁰ Dunn,²¹ Jacobs,²³ and their colleagues, obtained by the technique of determining changes in the specific activity of a trace dose of uniformly labeled glucose-C¹⁴ after insulin administration, indicate that the rate of insulin administration appears to affect the magnitude of the hepatic response. When insulin in large amounts is injected intravenously, there results only a transient decrease in hepatic glucose output and a prolonged increase in peripheral glucose utilization of considerable magnitude. By contrast, when insulin is administered subcutaneously or by slow intravenous infusion, a depression of hepatic glucose output of considerable duration ensues. In the present studies different parameters, the arteriovenous (A-V) glucose difference across the leg²⁴ and the hepatic output of glucose,²⁵ were measured during the slow infusion of glucagon-free insulin, and a similar depression of hepatic glucose output was found.

Changes in femoral A-V glucose difference were determined in 8 normal subjects during the intravenous infusion of glucagon-free insulin administered for 120 min. at a rate of 0.06 to 0.1 U./min. The changes in A-V glucose difference were then compared to changes that occurred in the same 8 subjects following the intravenous administration of 1 gm. of sodium tolbutamide. Femoral arterial and venous blood samples were collected simultaneously at 10-min. intervals during a 150- to 180-min. period by means of indwelling Cournand needles. Blood glucose concentrations were determined by the Somogyi copper iodometric method²⁶ on 2 ml. blood samples.

During insulin infusion mean arterial blood glucose concentration fell from 80 to 52 mg. per cent; femoral A-V glucose difference failed to increase and averaged only 1.8 mg. per cent. Similar results occurred after tolbutamide administration. Mean blood glucose concentration fell from 83 to 52 mg. per cent and A-V glucose differences averaged 2.3 mg. per cent. The data indicate no significant difference in peripheral glucose utilization, as manifest by changes in A-V glucose difference after tolbutamide and after the slow infusion of insulin. While it is possible that peripheral glucose utilization was increased but could not be detected after insulin administration, these data can be alternately interpreted to indicate an hepatic effect of both tolbutamide and insulin under these experimental conditions. Such an interpretation is in accord with the previously mentioned data of Jacobs *et al.*, who noted an hepatic effect of insulin administered subcutaneously or by slow intravenous infusion.²³ A typical set of experiments showing the effects on A-V glucose difference of insulin and tolbutamide are shown in FIGURES 1 and 2.

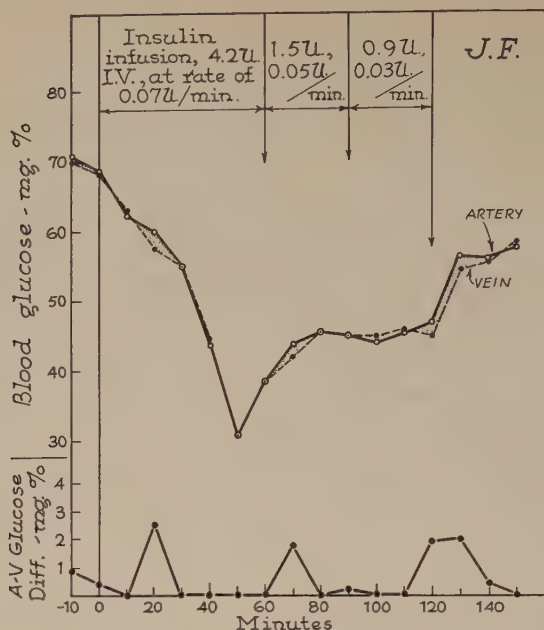


FIGURE 1. Typical example of the effects of the slow infusion of glucagon-free insulin on femoral arteriovenous glucose difference in normal subjects.

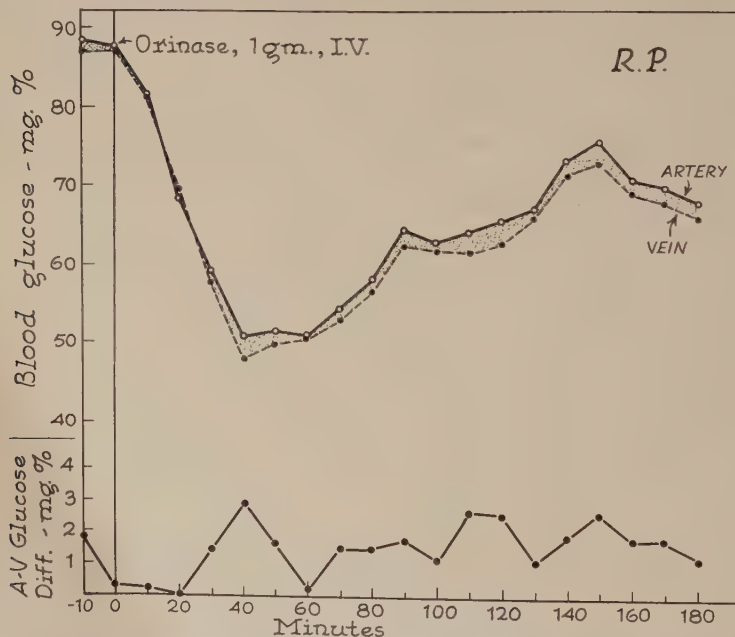


FIGURE 2. Typical example of the effects of the intravenous administration of 1 gm. of sodium tolbutamide on the femoral arteriovenous glucose difference in a normal subject.

In order to determine by direct measurement whether the slow infusion of insulin results in a decreased hepatic output of glucose, hepatic blood flow and hepatic vein-arterial glucose differences were measured in normal and diabetic subjects. Following hepatic vein catheterization, hepatic blood flow was measured by the Bromsulphalein technique of Bradley *et al.*²⁷ Simultaneous hepatic venous and arterial blood samples were collected at 10-min. intervals for 20 to 30 min. prior to and during the 60-min. duration of the insulin infusion. Examples of two experiments, one in a diabetic subject and the other in a normal subject, are shown in TABLE 1 and in FIGURE 3. In both instances the hypoglycemia following the insulin infusion was associated with a profound reduction in the hepatic output of glucose.

TABLE 1
EFFECT OF INSULIN INFUSION ON HEPATIC GLUCOSE OUTPUT IN A LABILE DIABETIC

Time (minutes)	Estimated hepatic blood flow (ml./min.)	Blood glucose (mg./100 ml.)			Hepatic glucose output (mg./min.)
		Hepatic vein	Femoral artery	HV-Art.	
-25	962	450	270	180	1730
-15	1144	475	269	206	2360
- 5	1177	427	274	153	1800
Start insulin infusion—0.1 U./min.					
10	1030	373	275	98	1010
20	1121	395	258	137	1540
30	970	345	250	95	922
40	929	301	230	71	660
50	907	311	221	90	820
60	895	210	207	3	27
70	883	210	185	25	222

In order to obviate the criticism that these changes reflect only alterations in net splanchnic glucose output and not necessarily in net hepatic glucose output, eight experiments were performed on dogs with complete portacaval shunts. In this preparation, all the blood perfusing the liver comes from the arterial circulation. A typical example of the effect of the infusion of 0.025 U./min. of glucagon-free insulin on hepatic venous and arterial blood glucose concentrations and on the hepatic output of glucose is shown in FIGURE 4. In this dog, mean hepatic glucose output prior to insulin infusion was 64 mg./min. and fell to 25 mg./min. during insulin administration. Concomitantly, arterial blood glucose concentration fell from 84 to 66 mg. per cent.

These data indicate that under proper experimental conditions exogenous insulin apparently has an effect upon hepatic glucose metabolism. Since all endogenous insulin must traverse the liver before entering the systemic circulation, the concentration and amount that reach the liver must exceed those reaching all other tissues. This suggests that endogenous insulin

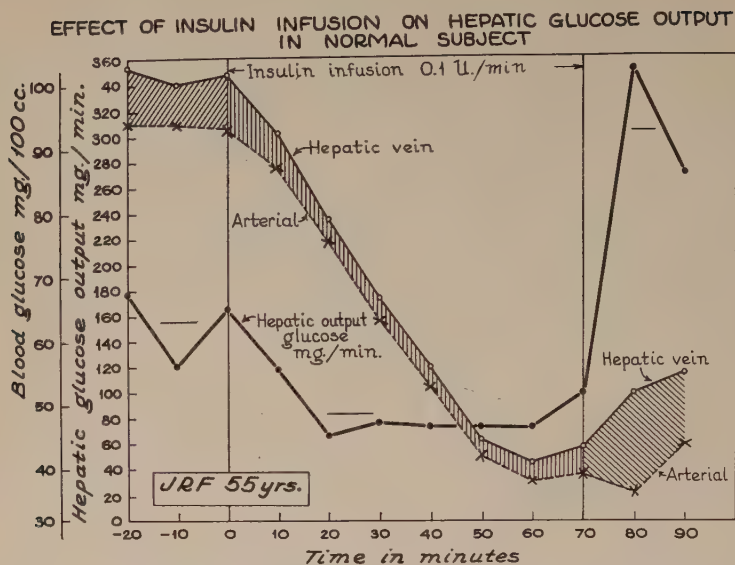


FIGURE 3. Effect of the slow infusion (0.1 U./min.) of glucagon-free insulin on the hepatic venous-arterial glucose difference and estimated hepatic output of glucose in a normal subject.

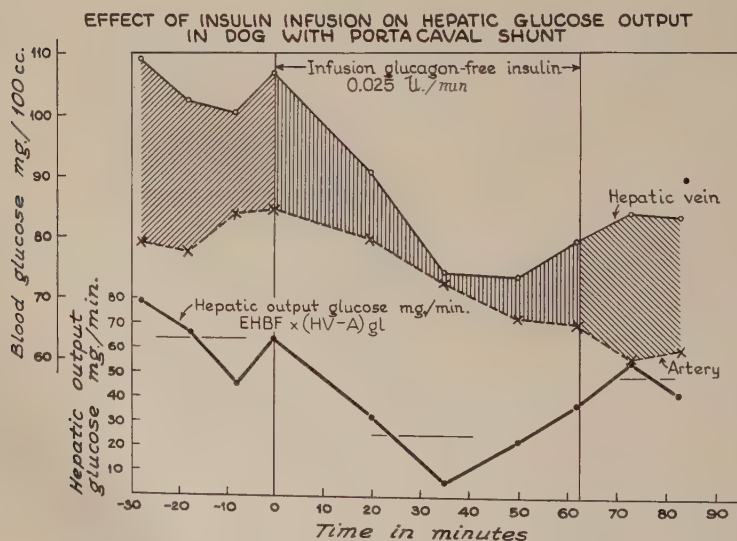


FIGURE 4. Effect of the slow infusion (0.025 U./min.) of glucagon-free insulin on the hepatic venous-arterial glucose difference and hepatic output of glucose in a dog with a complete portacaval shunt.

may have a greater hepatic effect than insulin administered into the systemic circulation and therefore may account for the hepatic effect of the sulfonylureas.

In order to determine whether the initial passage of insulin through the liver alters the magnitude of its hepatic and peripheral actions, the effects of glucagon-free insulin administered by the portal vein and by a peripheral vein were compared in 16 experiments on 8 dogs. The details of these experiments have already been reported.²⁸ In these studies, the magnitude of the arterial hypoglycemia was the same after both routes of administration. However, in each dog the peripheral injection of insulin resulted in a significantly greater increase in peripheral glucose utilization than did the portal administration. The combination of similar arterial hypoglycemia coupled with a lesser augmentation of peripheral glucose utilization after portal injection suggests that portal administration of insulin results in a greater effect on hepatic glucose metabolism than does the administration of insulin into a peripheral vein. Hagedorn and Poulsen have also reported a greater hepatic effect of insulin in rabbits when administered by the portal vein than when injected into the inferior vena cava.²⁹ A typical example of the comparative effects of the portal and peripheral routes of insulin administration on the magnitude of arterial hypoglycemia and on the magnitude of increase in peripheral glucose utilization is shown in FIGURE 5.

The apparently greater hepatic effect of insulin injected by the portal vein suggests that a substantial amount of this insulin is retained by the liver during the initial transhepatic passage. Studies were designed to determine whether a significant amount of insulin is retained by the liver during a single circulation and also to compare the peak hepatic binding of insulin after portal and peripheral administration.³⁰ In the first study, 196 rats were injected with I¹³¹-labeled insulin. In one half of the rats the insulin (0.002 units) was injected into the portal vein; in the other one half, into the femoral vein. The rats were sacrificed at frequent intervals from 4 sec. to 15 min. after insulin injection. Immediately thereafter the liver was perfused with 30 to 50 cc. of modified Ringer's solution. The liver, kidneys, and other tissues were then assayed for radioactivity in a well-type gamma counter and the percentage of the administered dose bound in each organ was determined. The results indicate that during the single transhepatic circulation following portal injection, 51 per cent of the administered insulin was bound firmly to the rat liver. By contrast, peak hepatic binding after the peripheral route of administration was only 27 per cent of the administered dose.

In order to determine whether the human liver binds a significant amount of insulin during a single transhepatic circulation, the following study was performed in 12 human subjects without known hepatic or pancreatic disease.³¹ During abdominal operation a solution containing 0.55 U. of I¹³¹-labeled insulin and a known amount of inulin was injected rapidly into the portal vein. Ten seconds later a brachial arterial blood sample was collected over a 10- to 15-sec. period. The concentrations of inulin and insulin-I¹³¹ were then determined. Since known amounts of insulin-I¹³¹ and inulin were

injected, their volumes of distribution could be calculated and compared. Control studies were performed in 8 subjects by injecting the solution of inulin and insulin- I^{131} into the antecubital vein and collecting the arterial blood sample at precisely the same time as after portal injection.

In the control studies the volumes of distribution of labeled insulin (2058 ml.) and inulin (1989 ml.) were almost identical. By contrast, after portal injection the volume of distribution of the labeled insulin (5581 ml.) was more than twice as large as that of inulin (2584 ml.). This indicates that 52 per cent of the insulin was bound to the human liver in a single transhepatic circulation.

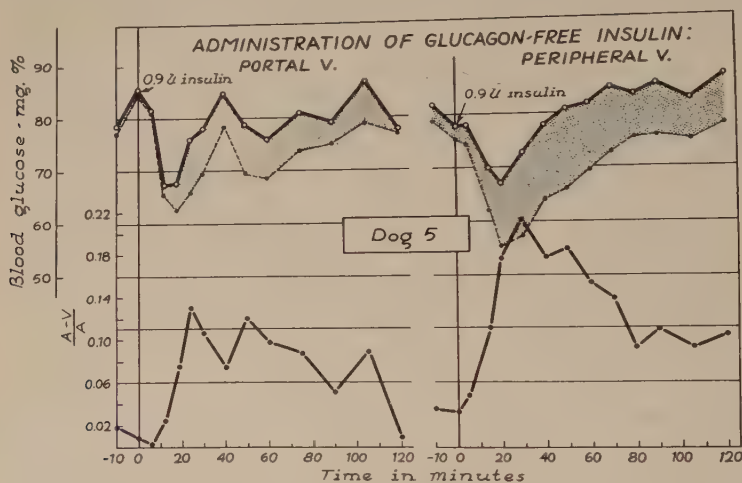


FIGURE 5. Typical example of the comparative effects of glucagon-free insulin administered by the portal vein and by a peripheral vein on the magnitude of the hypoglycemia and the magnitude of the increase in peripheral arteriovenous glucose difference. Note that the degree of hypoglycemia is the same after both routes of administration and that the arteriovenous glucose differences are greater after the peripheral administration of insulin. Reproduced by permission of *The Journal of Clinical Investigation*.²⁸

These data support but do not prove the concept that endogenous insulin in man regulates the hepatic output of glucose. However, they should not be construed to mean that this is the sole function of insulin. We have other data indicating that, when the hepatic insulin binding sites are saturated³² or blocked,³³ a greater portion of endogenous insulin passes through the liver to the periphery where it exerts a greater effect on peripheral glucose utilization.³⁴ Crandall and Cherry have also previously reported that changes in the hepatic output of glucose follow anesthesia and acute operative stress.³⁵

Summary and Conclusions

There is a growing body of evidence, not yet incontrovertible, indicating that insulin, when administered under proper experimental conditions, results in a decreased hepatic glucose output and thereby contributes to the

hypoglycemic effect. The data from the present experiments support such an action. Moreover, the data indicating that more than 50 per cent of labeled insulin injected intraportally is bound by the liver suggests that endogenous insulin may play a significant role in the regulation of the hepatic release of glucose.

While these findings do not prove the beta-cytotropic mechanism of action of the sulfonylureas, they are in complete accord with such a hypothesis. The failure of the sulfonylureas to duplicate precisely the effects of exogenous insulin may be related to the evidence presented here, which indicates that the site, rate, magnitude, and duration of insulin administration apparently alter its subsequent metabolic effect. For these reasons such differences cannot be used to controvert other formidable evidence indicating that the hypoglycemic action of the sulfonylureas is beta-cell dependent and that these agents increase the secretion of endogenous insulin.

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CHANGES INDUCED BY INSULIN AND TOLBUTAMIDE IN THE GLUCOSE OUTPUT OF THE LIVER

P. Schambye and F. Tarding

Research Laboratories, Novo Terapeutisk Laboratorium A/S, Copenhagen, Denmark

Following the independent observation of the additional hypoglycemic effect of the sulfonylureas in insufficiently insulin-treated pancreatectomized dogs¹ it became evident that these compounds might produce the decrease in the blood sugar concentration of both normal and diabetic dogs by inhibiting, either partly or totally, the release of glucose from the liver.

The first part of this report describes our experimental observations after applying the single injection of C¹⁴-labeled glucose for measuring the glucose output in the liver in normal dogs before and after intravenous injections of either tolbutamide or insulin.

A new method for calculating this output from the isotope data was developed. By equaling the ratio between specific activity of glucose in samples of hepatic venous and arterial blood to the amount of glucose entering and leaving the liver, the output of unlabeled glucose from this organ could be calculated for any period before or after the administration of a substance producing a change in the size of the glucose pool. This method may have an advantage over the ordinary isotope-dilution method based upon injection of C¹⁴-glucose, which does not allow calculation of the glucose turnover during the actual changes of the plasma glucose concentration, as the latter will not necessarily be representative of the various compartments of the glucose pool.

The second part of the report deals with the results obtained with this method when insulin was infused portally into normal, cyclopropane-anesthetized dogs.

Effect of Intravenous Injections of Tolbutamide or Insulin²

These studies were performed in normal dogs, fasted overnight and anesthetized with pentobarbital sodium (Nembutal); a dose of about 200 μ c. of uniformly labeled C¹⁴-glucose, representing about 2 mg. glucose, was injected intravenously. Following an equilibration for about 1 hour, blood samples were collected every 15 min. through a polyethylene catheter placed in a peripheral vein. After a control period of about 1 hour, a total dose of tolbutamide (0.15 gm./kg.) was injected intravenously in 2 doses (0.10 gm./kg. and 0.05 gm./kg., 10 min. apart) and blood samples were collected at intervals of 10 to 30 min. for the next 2 to 5 hours. As shown in FIGURE 1, the exponential decrease of the blood glucose specific activity observed in the control period, which reflected a constant release of unlabeled glucose from the liver to replace the amount of glucose utilized in the peripheral tissues, was interrupted by the injection of the tolbutamide, as the specific activity remained almost constant for a period of 30 to 40 min. During this time the plasma glucose concentration decreased to about 40 to 50 mg. per cent. When these hypoglycemic levels were reached, the specific activity

again decreased exponentially at a rate slightly higher than in the control period but nevertheless corresponding to a lesser release of glucose from the liver.

For the example shown in FIGURE 1 the rate of glucose release from the liver was about 57 mg./min. in the control period. Assuming that a similar

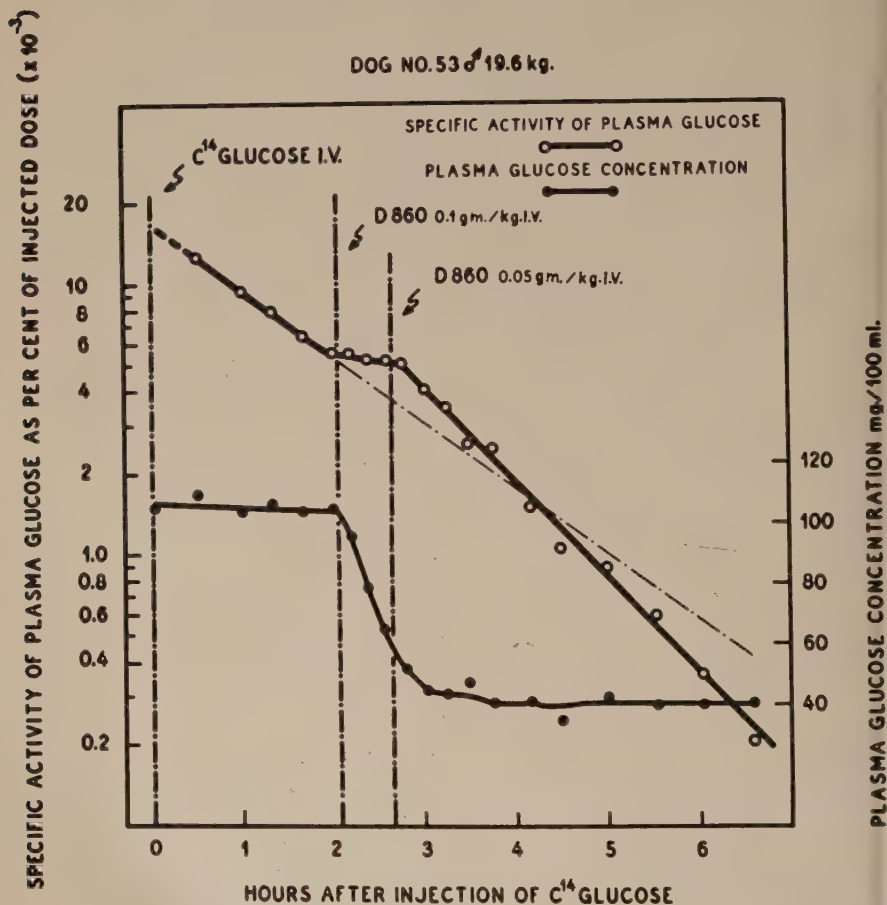


FIGURE 1. The effect of intravenously injected tolbutamide (D 860) on the plasma glucose concentration and specific activity in an anesthetized normal dog.

rate of peripheral glucose utilization persists under the actual decrease in the blood sugar, this rate would be sufficient to decrease the blood sugar to the level observed, as this would require a utilization of about 65 mg./min., which is not significantly different from the control value. The steeper decline later in the postinjection period, indicating the rate to be about 45 mg./min., does not reflect an increased rate of utilization, but is due to the considerably smaller glucose pool.

Searle and Chaikoff³ observed a similar plateau after creating a hyper-

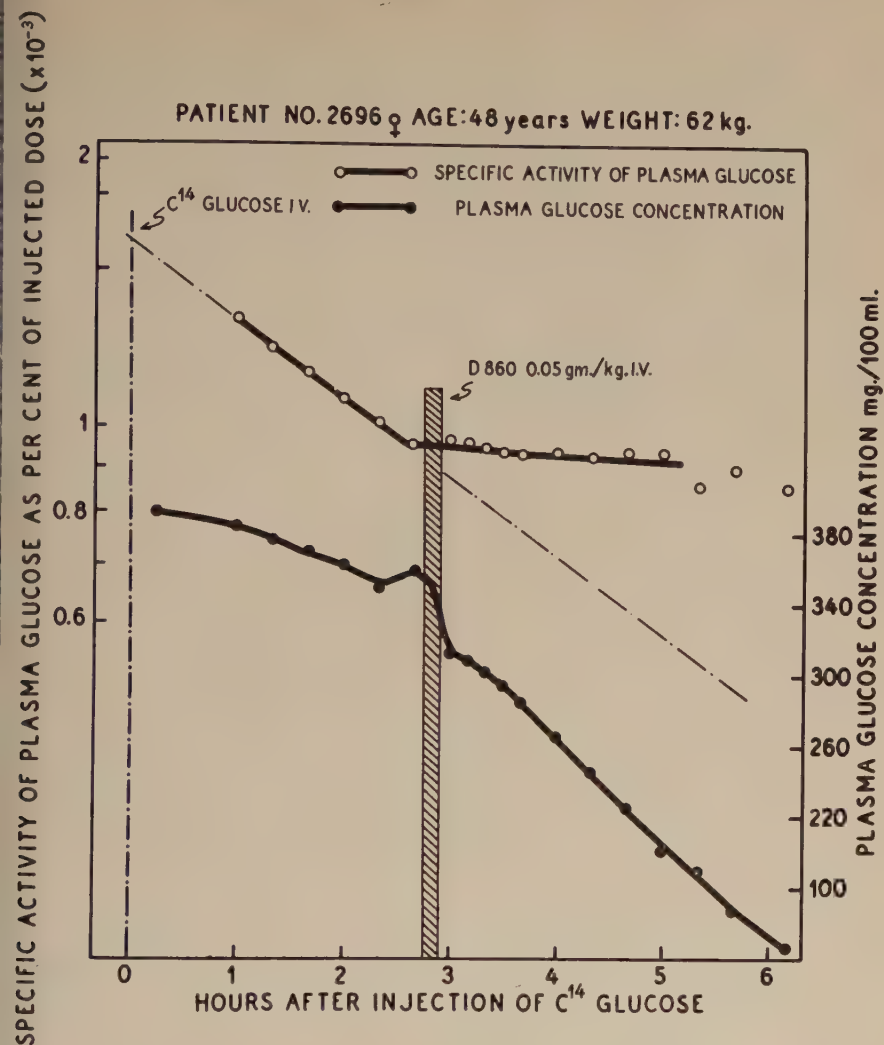


FIGURE 2. The effect of intravenously injected tolbutamide (D 860) on the plasma glucose concentration and specific activity in an unanesthetized diabetic patient who received the last injection of regular insulin 18 hours prior to the examination.

glycemia that was interpreted as an inhibition of the glucose output. According to this, the practically constant specific activity of the blood glucose after the injection of tolbutamide may also indicate a block in the glucose release from the liver. The short duration of a complete inhibition is thought to be due to the measures taken by the organism to overcome the large degree of hypoglycemia shortly after it has been established.

Actually, this explanation was indirectly confirmed, when a plateau in the specific activity curve was observed, under identical experimental conditions,

in 2 diabetic patients and 2 pancreatectomized dogs. In these cases the plateau lasted for the entire postinjection period of observation (FIGURES 2 and 3). Although exogenous insulin (20 I.U. lente insulin) had been administered to the diabetic dogs only 18 hours before the injection of tolbutamide, a very clear-cut inhibition of the glucose output of the liver was produced (FIGURE 3). As a result of this, the blood sugar decreased from about 550 mg. per cent to about 250 mg. per cent in the course of 4 hours. This decrease was produced by the rate of glucose uptake in the peripheral tissues and the excretion in the urine.

The experimental data of Creutzfeldt⁴ and others indicate that the hypoglycemia might be produced by a stimulation of the release of insulin from the beta cells of the Langerhans islets. Therefore, identical experiments were carried out, replacing tolbutamide with 2 intravenous injections of

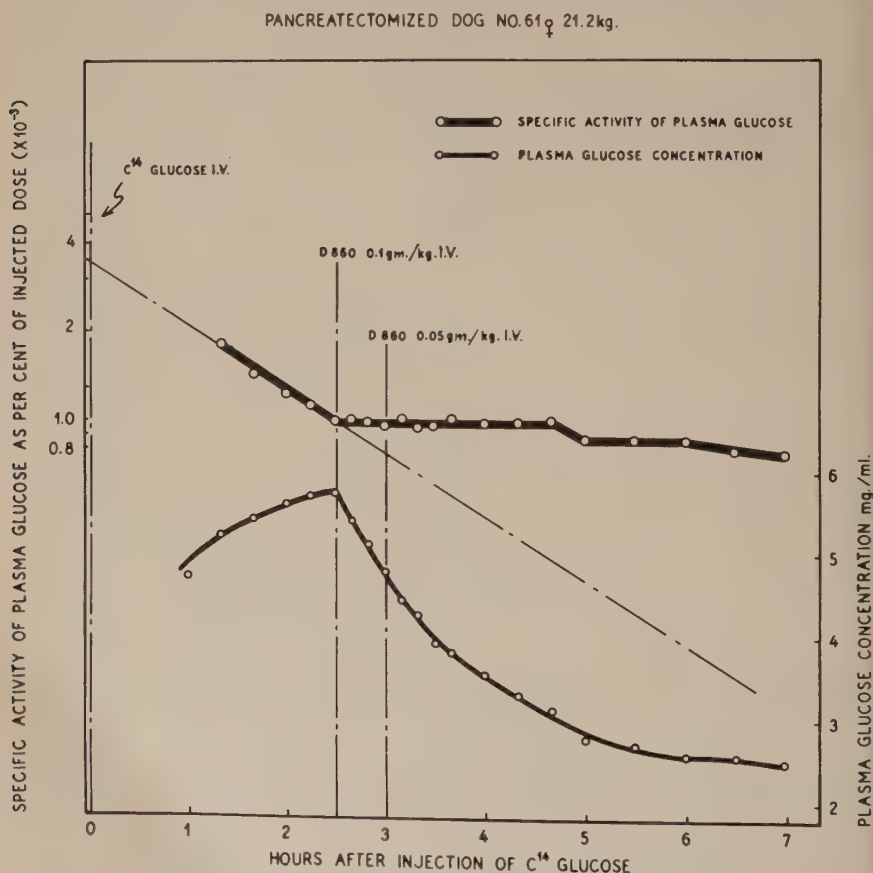


FIGURE 3. The effect of intravenously injected tolbutamide (D 860) on the plasma glucose concentration and specific activity in an anesthetized, pancreatectomized dog which, 18 hours before the injection of the C¹⁴-glucose, received its last dose of insulin (lente insulin 14 I.U. + insulin 6 I.U.).

insulin (0.1 I.U. and 0.05 I.U./kg. at 10-min. intervals). In contrast to the findings after tolbutamide, this was immediately followed by a considerably steeper decrease in the specific activity, which cannot be produced by an inhibition of the glucose release from the liver (FIGURE 4).

In an independent study Dunn *et al.*⁵ observed a very short plateau (5 to 10 min.) in the specific activity curve following doses of insulin in the range

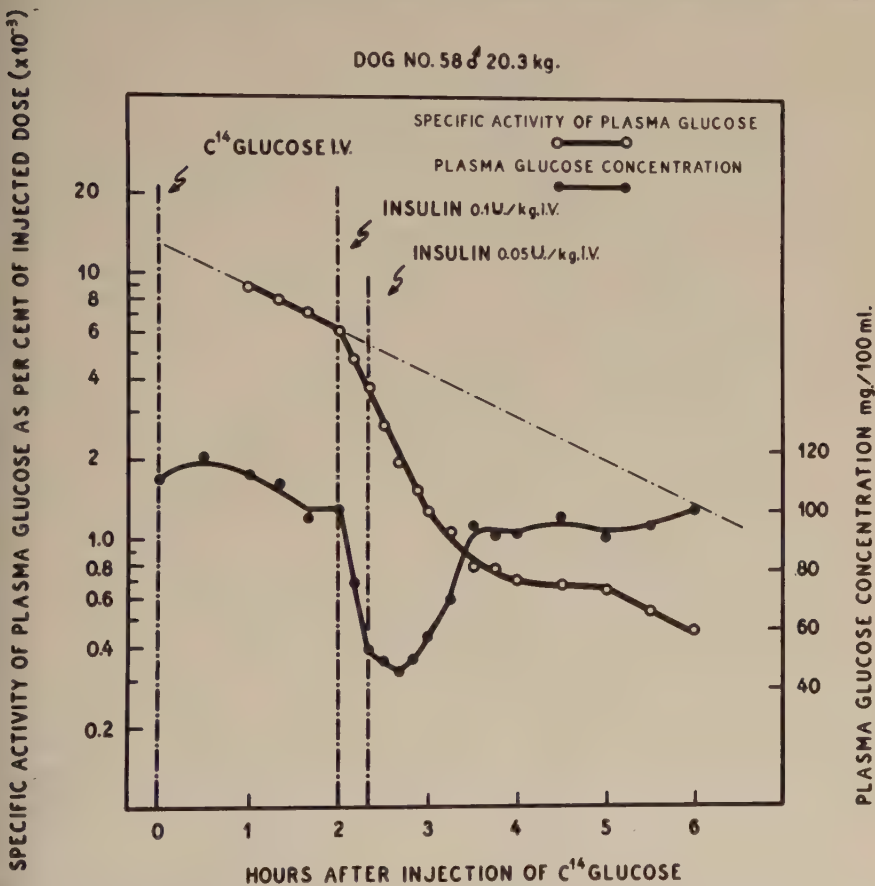


FIGURE 4. The effect of intravenously injected insulin on the plasma glucose concentration and specific activity in an anesthetized normal dog.

of 1 I.U./kg. To a certain extent we can confirm this observation, as doses of insulin exceeding 0.5 I.U./kg. produce what appears to be a very short-lasting plateau; indeed, far shorter than that observed after tolbutamide. This may be related to these rather unphysiological doses of insulin, especially as it is not observed in our experiments when smaller and perhaps more physiological doses of insulin are injected. Therefore, from our point of view, it is not likely that the hypoglycemic effect of tolbutamide is due to an enhancement of the insulin release from the pancreas.

However, in order to test the possibility that an increase of the insulin secreted from the pancreas into the portal blood might have a special and limited effect upon the glucose output from the liver, the subsequent study of the effect of insulin constantly infused intraportally in amounts varying from 0.02 to 0.12 I.U./kg./min. was undertaken.

The Glucose Output of the Liver Following Intraportal Insulin Infusions

The principles of the calculation of the amount of unlabeled glucose released from the liver are shown schematically in FIGURE 5. The specific activity

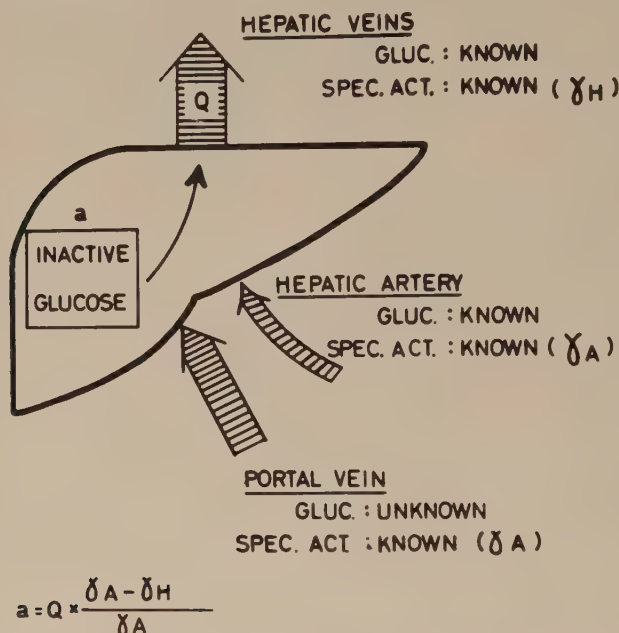


FIGURE 5. The principle of the isotope-dilution method worked out for calculating the release of unlabeled glucose (a) from the liver. (Q = total amount of glucose leaving the liver.)

of the glucose in portal and arterial blood is identical if no dilution occurs with unlabeled glucose absorbed from the intestines. This is confirmed experimentally in normal dogs starved for 18 hours, although the concentration of glucose may differ in blood from these two vessels. The arterial specific activity can thus be regarded as representative for all blood glucose entering the liver. If γ_A and γ_H represent the specific activity of glucose in arterial and hepatic-venous blood, the ratio γ_A/γ_H will be equal to the ratio between the amount of glucose leaving the liver (Q) and the amount passing through the liver (D). Hence:

$$\frac{\gamma_A}{\gamma_H} = \frac{Q}{D} \quad (1)$$

While D cannot be ascertained, the value of Q is calculated from the estimated hepatic blood flow⁶ and the hepatic-venous glucose concentration, and further, as

$$Q = D + a \tag{2}$$

where a is the amount of unlabeled glucose released from the liver, this amount can be derived as:

$$a = Q \left(1 - \frac{\gamma^H}{\gamma^A} \right) \tag{3}$$

This equation will be suitable for liver output calculations even in periods

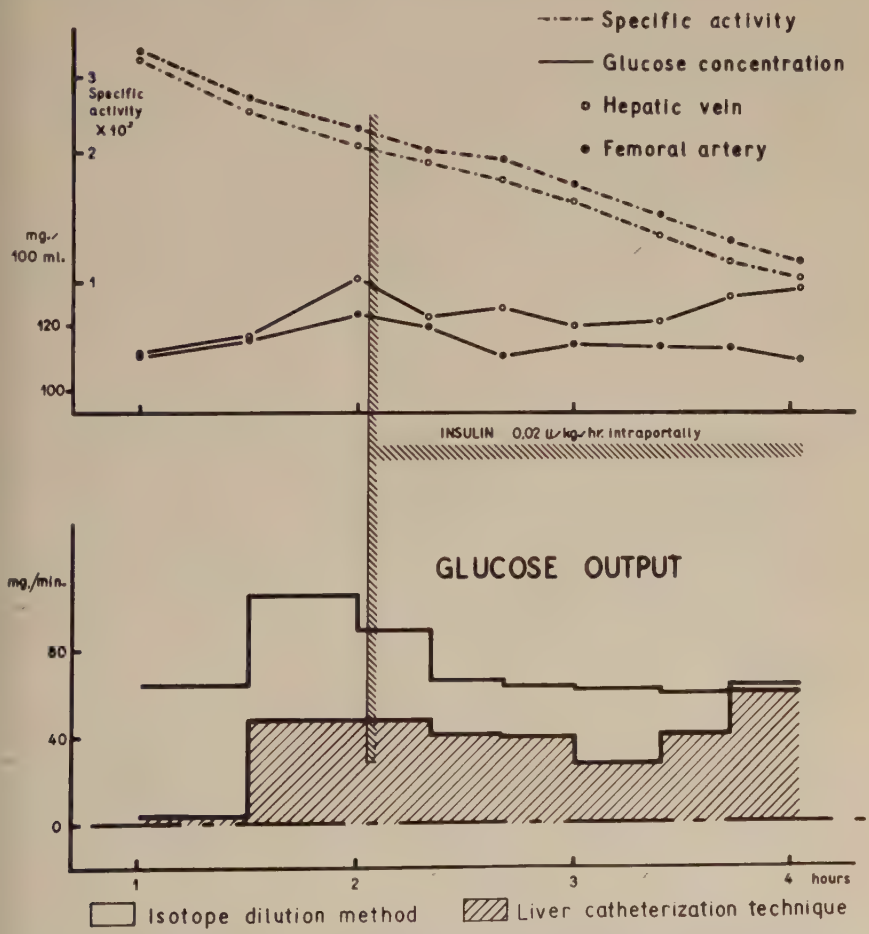


FIGURE 6. Effect of intraportal infused insulin (0.02 I.U./kg./hour) into a normal dog upon the output of unlabeled glucose from the liver, and the net amount of glucose leaving the splanchnic area. In the upper part of the figure are shown the arterial and hepatic-venous plasma glucose concentration and specific activity.

where a steady state with respect to glucose metabolism is not maintained, as variations in the glucose pool do not interfere.

The output of unlabeled glucose from the liver before and during an intra-portal infusion of 0.02 I.U. of insulin/kg./hour is shown in FIGURE 6, together with the net amount of plasma glucose leaving the splanchnic organs, calculated from the difference in glucose concentration of hepatic-venous and arterial plasma multiplied by the estimated hepatic plasma flow. Although this dose of insulin does not have any apparent effect upon the arterial blood sugar concentration, it could be anticipated that if insulin effects the glucose uptake of the liver cells, this dose given intraportally might be followed by an alteration in the net amount of glucose leaving the liver, and also in its release of unlabeled glucose. This, however, was not the case, as the values remained approximately constant for the whole infusion period, although the hepatic cells presumably were exposed to concentrations of insulin that would have stimulated the glucose uptake of peripheral tissues.

Similar results were obtained with intraportal infusion of 0.04, 0.08 and 0.12 I.U./kg./hour. The decrease in the arterial blood sugar indicates only an increased uptake of glucose in the peripheral tissues, as the output from the liver remained almost constant in this period. Thus, the results obtained after intraportal infusion of relatively small doses of glucagon-free insulin into cyclopropane-anesthetized normal dogs indicate that the glucose output from the liver is not, even partly, inhibited by the influence of exogenous insulin.

Discussion

The temporary stop in the decrease of the plasma glucose specific activity following tolbutamide injections into normal dogs and, more so, the longer lasting plateau observed in the pancreatectomized dogs are thought to be due to an inhibition of the output of unlabeled glucose from the liver. A similar inhibitory action of the sulfonylureas upon the hepatic release of glucose has been observed by Anderson *et al.*,⁷ Recant and Fischer,⁸ and Ashmore *et al.*⁹ Simultaneously, a stimulatory effect upon the glucose uptake of the peripheral tissues could not be demonstrated, which is in accordance with the findings of Berson and Yalow¹⁰ and of Ashmore *et al.*⁹

Following administration of insulin, either as a single intravenous injection or intraportal infusion, the decrease in the plasma glucose concentration was never associated with any decrease in the hepatic glucose output. Thus, the stimulatory effect of insulin upon the glucose uptake in the periphery was responsible for the fall in the blood sugar. This was also evident from the increased rate at which the plasma glucose specific activity decreased after the insulin was administered, and confirms previous observations with C¹⁴-glucose.^{9, 10}

Conclusions

As the hypoglycemic effect of the sulfonylureas is not associated with any such stimulation of the glucose uptake it must be concluded that their action is not likely to be exerted via an increase in the secretion of insulin from the pancreas or in the amount of insulin available in the body, but must be due

to its inhibitory effect upon the glucose output from the liver. However, as Dulin and Johnson¹¹ and Sobel *et al.*¹² have been able to demonstrate a hypoglycemic effect of sulfonylureas in the hepatectomized rat or dog, the action upon the hepatic glucose output may not be the only mode of action of these compounds, but it presumably plays a major role in the intact animal, which is not able to mobilize more liver glycogen to counteract the hypoglycemia produced by the sulfonylureas.

Summary

The influence of tolbutamide and insulin upon the hepatic glucose output and the glucose uptake in the peripheral tissues was studied in normal and pancreatectomized dogs and diabetic patients by applying the technique of single injection of C¹⁴-glucose.

Tolbutamide produced a definite cessation in the constant dilution of the plasma glucose specific activity normally caused by the release of unlabeled glucose from the liver to replace the blood sugar taken up by the periphery, as demonstrated by a plateau in the specific activity curve, which was most pronounced in the pancreatectomized dogs and the diabetic patients. As no increase in the peripheral utilization of glucose was demonstrated in these preparations, it was concluded that the hypoglycemic effect of tolbutamide is caused by an inhibition of the release of glucose from the liver.

By combining hepatic-venous catheterization with the C¹⁴-glucose technique a new method of calculating the hepatic glucose output was worked out; this was not affected by changes in the size of the glucose pool or the utilization of glucose in the splanchnic organs.

This technique was applied in a study of the effect of intraportal infusions of insulin into normal anesthetized dogs in doses ranging from 0.02 to 0.12 I.U./kg./hour. In no instance was this followed by a decrease in the hepatic glucose output; the hypoglycemia was solely produced by a stimulation of the peripheral glucose utilization, which is not observed after injections of tolbutamide.

It was therefore concluded that it is very unlikely that the sulfonylureas exert their hypoglycemic effect via an increased release of insulin from the pancreas.

Acknowledgments

The authors express their indebtedness to K. Hallas-Møller for many valuable suggestions and stimulating criticism, and to A. Thordahl-Christensen, The Royal Veterinary and Agricultural College, Copenhagen, for placing at their disposal the X-ray facilities of his department. The technical assistance of Kirsten Harder and of Lise Wollesen is also gratefully acknowledged.

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Discussion of the Paper

SIDNEY WEINHOUSE (*The Institute for Cancer Research, Philadelphia, Pa.*): I appreciate this opportunity of describing some experiments published in the September-October 1958 issue of *Diabetes*. These were carried out by my colleagues, Reichard, Jacobs, and Friedmann. Like the studies already reported by James Craig and Leonard Madison, they suggest an important role for insulin, as well as for the synthetic hypoglycemic agents, in suppressing hepatic glucose output.

FIGURE 1 shows results of an experiment similar to those that have just been described by Schambye, indicating that in the normal human hepatic glucose output is suppressed by the intravenous injection of insulin in a dosage of 0.1 U./kg. With the administration of insulin there is an immediate drop in the blood sugar, which coincides exactly in time with the "plateauing" of the specific activity. We interpret this plateau to indicate a suppression of glucose output, presumably from the liver. This is followed by a resumption in drop of specific activity at the time when the blood sugar begins to level off. Approximate inflow and outflow rates can be calculated from the data presented; this is shown in the inset of the figure. The solid line represents the inflow rates; the dotted line represents the rate of disappearance, presumably due to peripheral utilization. When the insulin is administered in one dose, there is a profound lowering or disappearance of glucose inflow simultaneous with a large increase in glucose disappearance rate.

FIGURE 2 shows a similar experiment in which tolbutamide was administered. Experiments were not carried out with chlorpropamide, unfortunately, but we hope to do this. Here again it can be seen that, coinciding with the hypoglycemic action of the drug, there is a "plateauing" of specific activity of the blood glucose for a period of about 20 to 30 min., again indi-

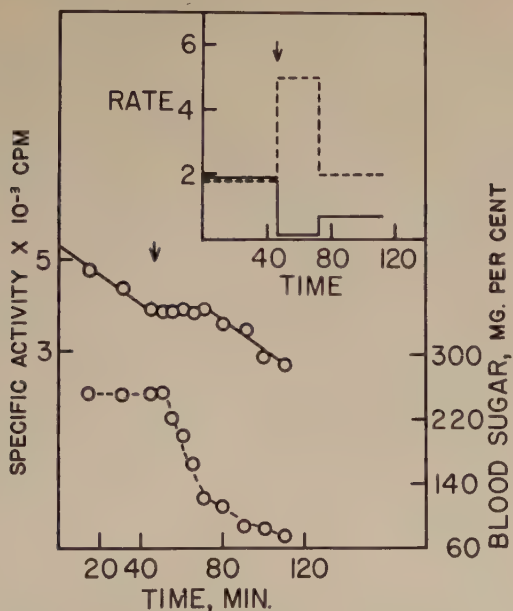


FIGURE 1. Blood sugar changes in a diabetic patient weighing 60 kg. and given 6 U. of insulin (indicated by arrow). Broken line represents blood glucose level; solid line, specific activity. Inset shows approximate values, with solid line representing entry; broken line, removal.

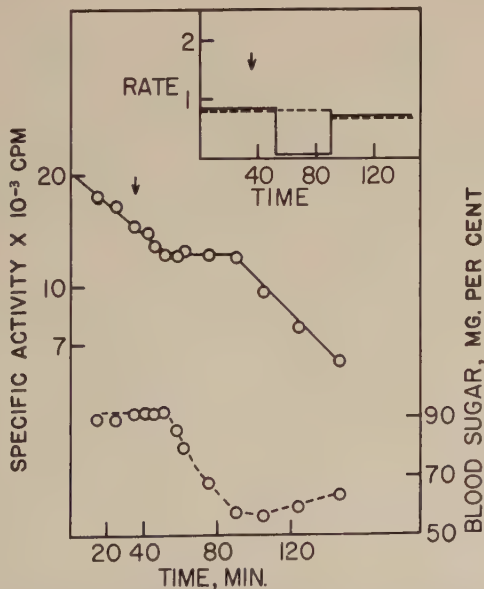


FIGURE 2. Blood sugar changes in a nondiabetic patient weighing 50 kg. and given 1.2 gm. tolbutamide intravenously (indicated by arrow). Broken line represents blood glucose level; solid line, specific activity. Inset shows approximate rates, with solid line representing entry; broken line, removal.

cating a suppression of glucose output. However, when the inflow and outflow rates are calculated, we find a significant difference from the experiments with insulin.

Although tolbutamide lowers the inflow of blood glucose, it does not have the stimulating effect on utilization observed when insulin was given. This apparent lack of stimulation of peripheral utilization represents a profound difference in the action of the two hypoglycemic agents.

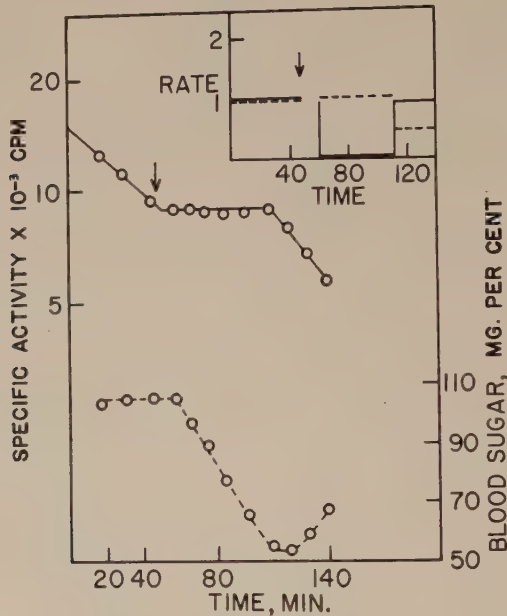


FIGURE 3. Effect on the time course of blood glucose concentration of 10 U. of insulin given subcutaneously to a nondiabetic patient weighing 66 kg. Arrow indicates time of administration of insulin. Broken line represents blood glucose level; solid line, specific activity. Inset shows approximate rates, with solid line representing entry; broken line, removal.

It also occurred to us, as it did to other investigators, that endogenous insulin secreted slowly into the portal vein might have an effect different from that of intravenously injected insulin. To simulate the slow release of insulin that, presumably, takes place when tolbutamide is injected, we did some experiments in which we injected the same amount of insulin subcutaneously, so that there would be slow release into the blood stream. FIGURE 3 shows the results of such experiments. Qualitatively, the picture is the same, but in this case, when insulin is administered in this fashion, we get the same result as with tolbutamide, namely, a profound lowering of inflow without a simultaneous increase in the utilization of glucose.

We interpret these results as indicating that the slow introduction of small doses of insulin into the blood stream causes a depression of the hepatic glucose output, but has little or no effect on the peripheral utilization of

glucose. We have also carried out experiments using slow intravenous infusion, and we have obtained identical results.

As others have indicated, these results do not exclude the possibility that tolbutamide or chlorpropamide have effects other than the stimulation of insulin release, but they do reconcile some of the previous results that were interpreted as indicating a different effect. More significantly, these results suggest to us that we should reconsider and re-evaluate the possibility of hepatic glucose output regulation in the problem of diabetes.

COMPARISON OF SOME METABOLIC EFFECTS OF INSULIN, CHLORPROPAMIDE, AND OTHER HYPOGLYCEMIC SUBSTANCES*

Piero P. Foà, Giorgio Galansino, Giuseppe D'Amico, Doris Kanameishi
Department of Physiology and Pharmacology, The Chicago Medical School, Chicago, Ill.

Convincing experimental evidence suggests that carbutamide and tolbutamide stimulate the release of insulin from the pancreas.¹⁻¹⁶ However, this cannot be accepted as the sole explanation for the hypoglycemia because these drugs do not share other metabolic effects of exogenous insulin.⁸⁻²¹ Two hypotheses may explain these differences in mode of action: (1) insulin secreted into the portal system as a result of normal pancreatic function or pancreatic stimulation acts differently from insulin injected peripherally; or (2) the drugs act not only on the pancreas, but on the liver^{13, 22} or other tissues also. Experiments designed to study these hypotheses have given contradictory results. Two groups of workers compared the effects of insulin and tolbutamide on the rate of disappearance from the plasma of injected C¹⁴-glucose. The first group²³ concluded that tolbutamide causes hypoglycemia by blocking the release of glucose from the liver, while insulin acts primarily by increasing peripheral glucose utilization. No qualitative differences were noted between the effects of insulin infused intraportally and of insulin injected into the femoral vein. The other group of workers²⁴ confirmed the observation concerning tolbutamide and insulin injected intravenously but, in addition, they found that, when insulin is injected subcutaneously and is slowly absorbed, it acts like tolbutamide, causing a suppression of glucose entry into the blood without affecting its removal. Still another group of workers²⁵ reported that, at comparable levels of hypoglycemia, injections of insulin into the portal vein are followed by smaller increases in peripheral glucose utilization than follow injections into a peripheral vein, thus confirming the observation that the metabolic effects of insulin may vary, depending upon the route of administration. Unfortunately, these conclusions are based on the measurement of arteriovenous (A-V) differences without measurements of blood flow. Experiments performed in this laboratory^{26, 27} did not disclose significant qualitative differences between the effects of insulin injected into the portal and insulin injected into the femoral vein of anesthetized dogs. In all cases insulin caused a decrease in the concentration of blood glucose and plasma potassium and an increase in the concentration of blood pyruvate and lactate. On the other hand, the intravenous administration of carbutamide and tolbutamide caused a decrease, not only in blood glucose, but also in blood pyruvate, lactate, and possibly in plasma potassium. Considering the numerous reports that the drugs are ineffective in the total absence of functioning pancreatic tissue, these results suggest that carbuta-

* The work reported in this paper was supported in part by Research Grant A-522 and Training Grant 2A-5102, National Institute of Arthritis and Metabolic Diseases, Public Health Service, Bethesda, Md.

mide and tolbutamide may act on the liver, but only if small (permissive) quantities of endogenous or exogenous insulin are provided.^{26, 27} Essentially the same conclusion was reached recently by other investigators.²⁸ This paper reports similar observations on the action of chlorpropamide.

Methods

Seventeen mongrel dogs of both sexes were fasted for 16 to 18 hours and anesthetized with a mixture of sodium pentobarbital (35 mg./kg.) and sodium barbital (100 mg./kg.), intravenously. This anesthetic mixture provided the rapid induction and the long-lasting, uniformly deep anesthesia with complete muscle relaxation that is essential to prevent fluctuations in blood pyruvate and lactate unrelated to the experiments. Chlorpropamide* was injected rapidly into the femoral vein in doses of 5 or 50 mg./kg. or into the pancreatic artery in doses of 5 mg./ml./kg., at the rate of 1 ml./min. Control injections of saline into the femoral vein and of saline or glucose (0.5 gm. in 20 cc.) into the pancreatic artery had been performed previously.^{27, 29} Blood samples were taken from an exposed femoral vein for duplicate analyses. Glucose was determined according to Nelson,³⁰ pyruvate according to Friedemann and Haugen,³¹ lactate according to Barker and Summerson,³² and potassium with the Coleman flame photometer. The carotid pressure of the dogs was used to check their general condition, which remained satisfactory in all but two animals; these were discarded. The statistical significance of the results was calculated according to Fisher.³³

Results and Discussion

The results are given in the form of curves representing average changes from preinjection values. FIGURE 1 shows that the injections of saline or chlorpropamide in doses of 5 mg./kg. into the pancreatic artery or into the femoral vein had no significant effect on peripheral blood glucose concentration. In contrast, the injection of glucose caused a significant ($p < 0.01$) hypoglycemia. The results suggest that, contrary to the results obtained under comparable conditions with carbutamide and tolbutamide, chlorpropamide does not stimulate insulin release. FIGURE 2 shows that the injection of chlorpropamide in doses of 50 mg./kg. into the femoral vein causes a prompt and significant decrease in the concentrations of blood glucose ($p < 0.01$) and pyruvate ($p < 0.01$); it also caused a delayed but significant ($p < 0.05$) decrease in the concentration of blood lactate and had no significant effect on the concentration of plasma potassium. This lowering of blood lactate and pyruvate may be the result of a decrease in the amount of glucose made available to the muscle, a fact that, in the fasted animal, most probably is due to decreased glucose production by the liver. The experiments were not designed to determine whether the hypoglycemic action of chlorpropamide, like that of carbutamide and tolbutamide, requires the presence of small (permissive) quantities of insulin.

* Donated by Chas. Pfizer & Co., Inc., Brooklyn, N. Y.

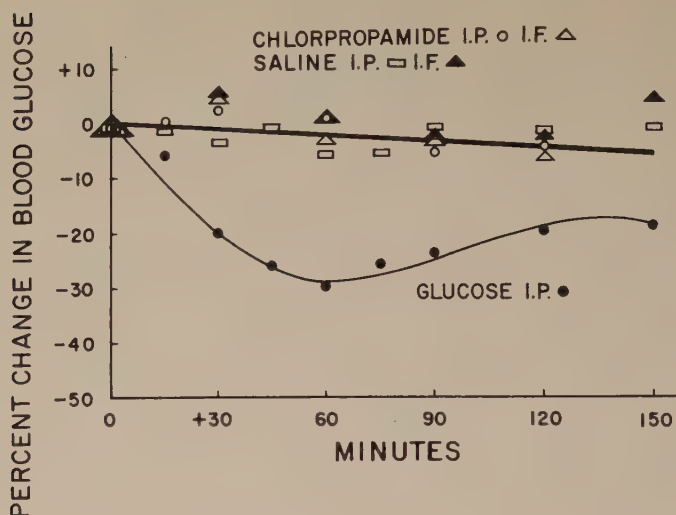


FIGURE 1. Effect of intrapancreatic (I.P.) or intrafemoral (I.F.) injections of chlorpropamide or saline (straight line) and of intrapancreatic injections of glucose (curve) on blood glucose.

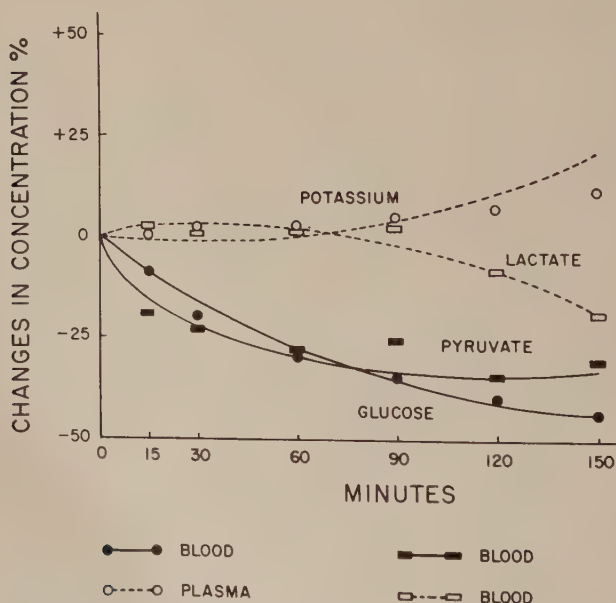


FIGURE 2. Effect of intrafemoral injections of chlorpropamide on blood glucose, pyruvate, lactate, and plasma potassium.

Summary and Conclusions

Chlorpropamide was injected into the pancreatic artery or into the femoral vein of anesthetized dogs. No significant changes in systemic blood glucose were noted following intrapancreatic or intrafemoral injections of chlorpropamide in doses of 5 mg./kg. On the other hand, intrafemoral injections of chlorpropamide in doses of 50 mg./kg. caused a decrease in the concentrations of blood glucose, pyruvate, and lactate. The results are consistent with the hypothesis that the hypoglycemic effect of the drug is due, at least in part, to a decreased liver glucose production and offer no evidence for a pancreatic action of chlorpropamide.

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EFFECT OF CHLORPROPAMIDE ON INTERMEDIARY METABOLISM IN HUMAN SUBJECTS*

Harold L. Dobson, Hooshang Guilak,† James A. Greene

Baylor University College of Medicine, and the Jefferson Davis Hospital, Houston, Texas

Development of the oral hypoglycemic agents has stimulated widespread investigation in an attempt to clarify their mode of action. As a consequence, the metabolic activities of insulin have been investigated more intensively. At the present time there are two general concepts about the action of the sulfonylureas. One concept is that the principal action of tolbutamide and carbutamide is to stimulate insulin release from the pancreas;^{1, 2} the other is that these agents produce hypoglycemia by decreasing hepatic glucose output without increasing peripheral utilization.³⁻⁷ Almost all metabolic studies reported in human and in experimental animals have been acute experiments; that is, the metabolic effects have been studied over a period of a few hours after the administration of the initial dose of the drug. It was felt that it would be worthwhile to investigate the metabolic effects of chronic administration of chlorpropamide in diabetic patients who were being maintained on it.

Methods and Materials

Diabetic patients were selected from the general Diabetic Clinic at Jefferson Davis Hospital, a city-county charity institution. Patients known to be free of metabolic disease and members of the professional staff served as normals. Where possible, studies were performed before as well as after chlorpropamide therapy was instituted. Each patient had received chlorpropamide at least one week before the "on treatment" study was performed. After an overnight fast the patients came to the metabolic laboratory and remained at rest in the laboratory until completion of the test. They took insulin and/or chlorpropamide in the dose of 500 to 1500 mg./day in their usual manner. Blood samples were obtained while the patient was fasting and at 1, 2, and 4 hours after administration. It is known that the composition of the diet will influence the blood levels of certain metabolites. It would be desirable to have all patients on a standard diet, but this is not possible because of the very limited resources available to this group of patients. Experience with these subjects has shown that the majority consume a rather constant diet that is low in protein and high in carbohydrate, fat, and calories;⁸ for this reason no dietary instructions were issued and the patients were asked to remain on their usual diets.

In the majority of published studies glucose has been used as the test meal. In order to determine whether any other abnormalities would appear when a

* The work reported in this paper was supported in part by a grant from Chas. Pfizer & Co., Inc., Brooklyn, N. Y.

† Trainee in Diabetes, Training Grant Program, National Institute of Arthritis and Metabolic Diseases, Public Health Service, Bethesda, Md.

more normal dietary mixture was used, the bulk of the data herein reported was obtained using a test breakfast containing 20 gm. protein, 65 gm. carbohydrate, and 20 gm. fat. Additional studies utilizing glucose have also been performed for comparison. Analyses were performed for blood sugar,⁹ inorganic phosphate,¹⁰ pyruvate,¹¹ alpha-ketoglutaric acid,¹¹ lactate,¹² total ketones,¹³ and citrate.¹⁴

Results

The blood sugar-lowering effect of chlorpropamide is seen in FIGURE 1. It should be noted that after the test meal the normals did not develop any elevation in blood sugar, which is probably due to delayed absorption. In contrast the diabetic developed significant elevations, both before and after

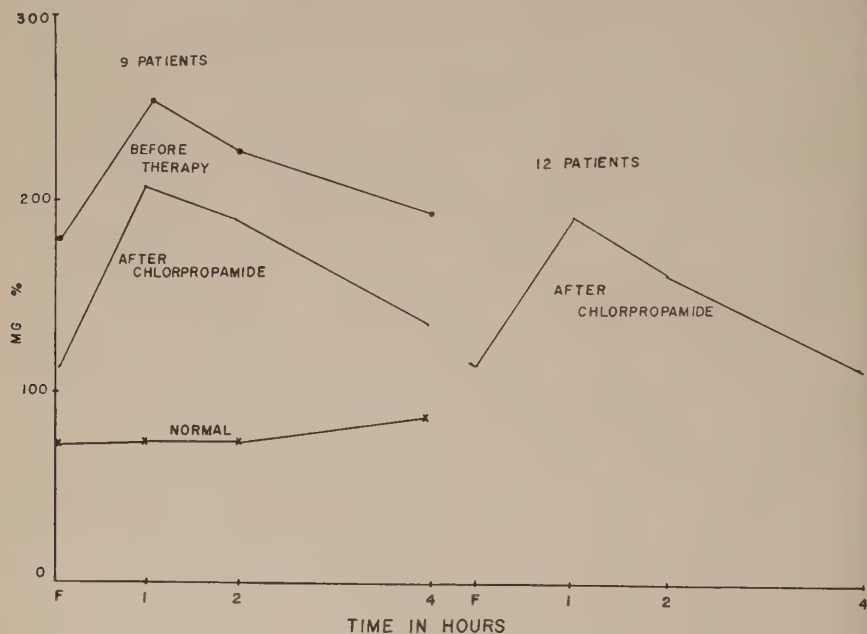


FIGURE 1. Blood sugar after test meal.

therapy. However, the after-therapy curve is significantly lower than the before-therapy curve, although its shape is not altered greatly. In general, these results are in agreement with those reported by other investigators utilizing tolbutamide and carbutamide in acute experiments.³⁻⁷

The studies on serum inorganic phosphate appear in FIGURE 2. In spite of the flat glucose tolerance curve, the normals showed typical fall in phosphate following the meal, and the values returned to normal by the fourth hour. In the insulin-treated diabetics this response is still seen, but is less marked. In the chlorpropamide-treated diabetics there is no change in serum phosphate level following the test meals. Statistical analysis indicates that there is no difference in the results obtained before and after therapy. These find-

ings are in agreement with other reports^{3, 5-7} on the effect of tolbutamide and carbutamide after acute administration.

Pyruvate blood levels (FIGURE 3) normally rise after a meal with fall back to normal at 2 to 4 hours.^{15, 16} The same is true of the insulin-treated diabetic. The untreated diabetic also has essentially the same response. The chlorpropamide-treated diabetic, however, has an elevated fasting level of pyruvate that fell after ingestion of the meal. This elevation is of borderline significance and appears in all of the experiments. This fasting elevation of pyruvate in chronically treated patients has not been reported previously. The data of Hennes *et al.*¹⁶ are not comparable, as these authors' subjects were

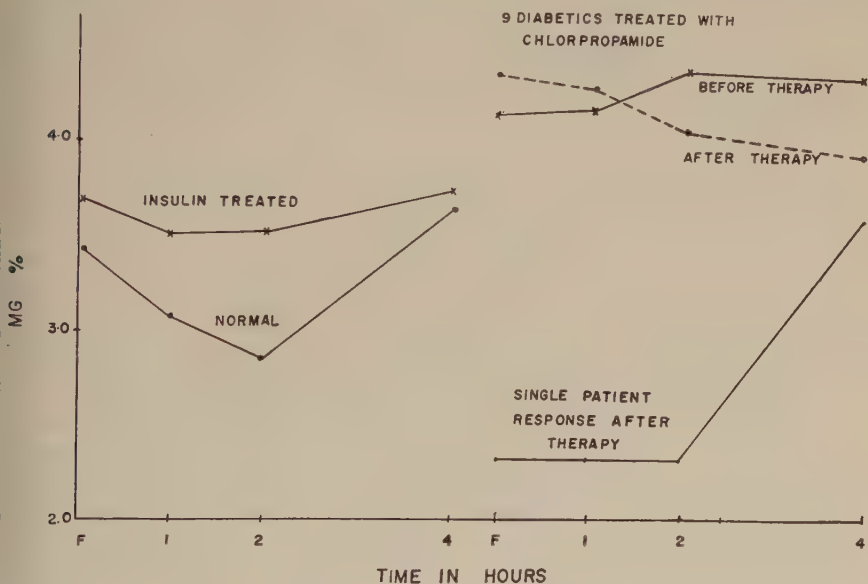


FIGURE 2. Inorganic phosphate.

not receiving sulfonylureas on a chronic basis. The elevation in pyruvate, if substantiated, would indicate either increased production, probably from gluconeogenesis or decreased utilization. As there has been no evidence that sulfonylureas increase gluconeogenesis, it is probable that the elevated fasting pyruvate level is due to decreased utilization of pyruvate.

Under the conditions of these studies pyruvate, lactate, citrate, and alpha-ketoglutarate will vary considerably, and alterations would require rather considerable magnitude to be significant. The results on lactate appear in TABLE 1 and show that there is no significant alteration, although there is considerable variability. The relatively high fasting values are probably due to recent activity of the patient in coming to the hospital rather than any drug-induced effect.¹⁷ In TABLE 2, it is seen that after the test meal the citric acid level rises at the first hour and then falls to normal. Other investigators have shown that the response of serum citrate in normal persons

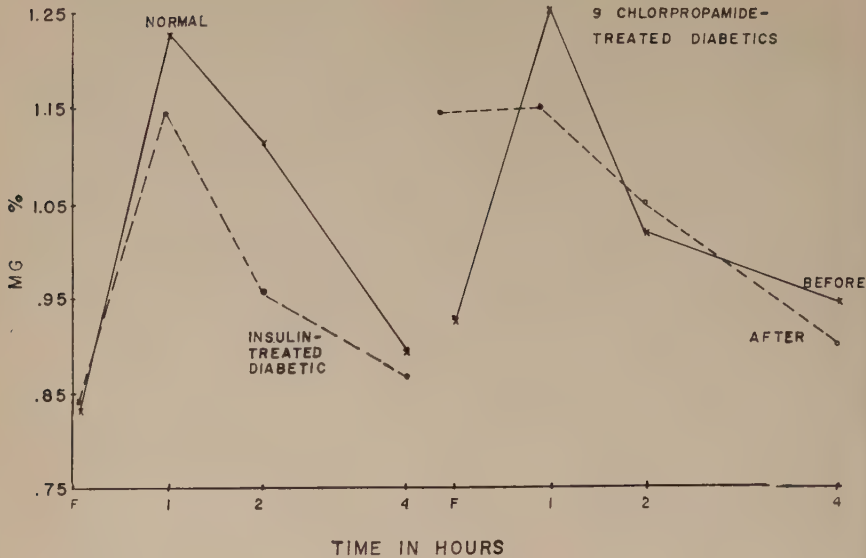


FIGURE 3. Pyruvate.

after glucose feeding is a fall with a return to normal in 4 to 6 hours.^{15, 18} However, in diabetics there is often a rise after the meal. Glucose was fed to 6 normal individuals, and the serum citric acid levels fell as described in the literature.¹⁸ It appears that the rise in citrate in normals after the test meal is related to the diet. In diabetics, before treatment, the postprandial rise in citrate is rather large and prolonged, and this is not altered after treatment with either insulin or chlorpropamide. The studies on alpha-ketoglutarate (TABLE 3) reveal no striking changes. The apparent elevated fasting levels after chlorpropamide therapy in the group that was studied before and after therapy would appear to approach significance, but the results obtained in the group studied only after chlorpropamide therapy do not support this.

TABLE 1
LACTATE
(Time in hours)

	F	1	2	4	No. pts.
	Mean ± S.E., mg. %				
Normal.....	11.1 ± 1.2	9.3 ± 0.62	9.3 ± 0.68	9.5 ± 1.13	6
Insulin.....	11.8 ± 1.3	12.9 ± 1.7	11.3 ± 1.5	11.8 ± 1.9	8
Chlorpropamide					
Before.....	10.9 ± 1.1	12.04 ± 1.3	12.5 ± 1.9	10.6 ± 1.4	12
After.....	9.9 ± 0.58	10.4 ± 0.66	10.8 ± 0.91	10.0 ± 0.75	15

TABLE 2
CITRIC ACID
(Time in hours)

	F	1	2	4	No. pts.
	Mean \pm S.E., mg. %				
Normal.....	2.1 \pm 0.28	2.2 \pm 0.16	2.01 \pm 0.24	1.82 \pm 0.13	6
Insulin.....	2.1 \pm 0.13	2.8 \pm 0.19	2.9 \pm 0.22	2.6 \pm 0.23	8
Chlorpropamide					
Before.....	2.6 \pm 0.08	3.0 \pm 0.25	3.2 \pm 0.15	2.6 \pm 0.10	9
After.....	2.3 \pm 0.12	2.9 \pm 0.24	2.8 \pm 0.14	2.5 \pm 0.23	9

TABLE 3
ALPHA-KETOGLUTARIC ACID
(Time in hours)

	F	1	2	4	No. pts.
	Mean \pm S.E., mg. %				
Normal.....	0.45 \pm 0.09	0.52 \pm 0.18	0.35 \pm 0.15	0.36 \pm 0.14	6
Insulin.....	0.47 \pm 0.10	0.69 \pm 0.12			13
Chlorpropamide					
Before.....	0.41 \pm 0.06	0.51 \pm 0.07	0.40 \pm 0.05	0.36 \pm 0.07	9
After.....	0.54 \pm 0.13	0.48 \pm 0.05	0.46 \pm 0.05	0.40 \pm 0.05	9
After-unpaired.	0.44 \pm 0.07	0.45 \pm 0.03	0.35 \pm 0.06	0.33 \pm 0.05	12

TABLE 4
KETONES
(Time in hours)

	F	1	2	4	No. pts.
	Mean \pm S.E., mg. %				
Normal.....	1.95 \pm 0.19	1.75 \pm 0.48	2.17 \pm 0.5	2.9 \pm 0.93	6
Insulin.....	3.15 \pm 0.56	2.24 \pm 0.49	2.75 \pm 0.66	2.8 \pm 0.59	8
Chlorpropamide					
Before.....	4.62 \pm 1.04	3.04 \pm 0.61	2.83 \pm 0.50	3.13 \pm 0.68	12
After.....	2.95 \pm 0.77	2.20 \pm 0.31	2.08 \pm 0.26	2.31 \pm 0.30	12

The blood ketone levels appear in TABLE 4. It may be seen that there is increased ketone production in the untreated diabetic. This is returned to a more normal level by the administration of insulin or chlorpropamide. The insulin-treated diabetics herein reported were in only fair control, and the chlorpropamide-treated patients had been on this drug for approximately 1 to 2 weeks. With better control of the disturbed carbohydrate metabolic defect in diabetics, the production of ketones is further diminished: for example, a study of 6 patients who were in excellent control after several months of chlorpropamide therapy showed a mean fasting blood ketone level of 0.89 ± 0.18 (S.E.). This does indicate that the chlorpropamide has the ability to increase the utilization of carbohydrate to the point of reducing ketone production to low normal.

Conclusions

The study of human intermediary metabolism in normal and diabetic patients before and after administration of insulin and chlorpropamide indicate that there are some differences between the metabolic action of insulin and chlorpropamide. The configuration of the glucose curve and the failure of the phosphate response indicate that chlorpropamide, on chronic administration, has essentially the same action as that of tolbutamide and carbutamide, and that this action differs from that of insulin. In addition, there is an elevation of fasting pyruvate level after the administration of chlorpropamide. The chronic administration of chlorpropamide effectively suppresses overproduction of ketones. No significant alterations were seen in citrate, lactate, and alpha-ketoglutaric blood levels; however, small differences would not appear under the conditions of these experiments.

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METABOLIC EFFECTS OF CONTINUED ADMINISTRATION OF SULFONYLUREA DERIVATIVES IN SELECTED DIABETIC SUBJECTS: INTERRELATIONS WITH GLUCAGON*

Joseph L. Izzo

Department of Medicine, University of Rochester School of Medicine and Dentistry, and Strong Memorial Hospital, Rochester, N. Y.

Although the mode of action of sulfonylureas is not completely understood, certain well-established facts have emerged from the accumulated clinical and experimental data. For example, several laboratories have demonstrated that the hypoglycemic action of sulfonylurea compounds is associated with a reduced output of glucose by the liver.¹⁻⁵ The mechanisms by which the reduced glucose output is accomplished have not been clearly established despite an intensive study of the problem. However, a decreased conversion of galactose or fructose to glucose by the liver has been observed during the period of hypoglycemia induced by administration of sulfonylureas,^{6, 7} and this has suggested the possibility that selective interference with hepatic gluconeogenesis might contribute significantly to the hypoglycemia. Little, if any, information is available on the effect of sulfonylureas on conversion of amino acids to glucose in the liver.

In the present studies an attempt has been made to determine whether the sulfonylureas might inhibit hepatic gluconeogenesis from protein and amino acids, as well as hepatic glycogenolysis. To this end, the effect of sulfonylureas on the metabolic responses to repeated daily intramuscular injections of glucagon has been studied. The ability of glucagon to increase the daily urinary nitrogen excretion as well as to raise the daily blood and urinary sugar levels with and without the simultaneous administration of carbutamide has been studied in two diabetic patients† who exhibited a striking hypoglycemic response to carbutamide. The effects of glucagon and carbutamide on the daily fasting levels of serum inorganic phosphorus, on the daily urinary excretion of reducing corticosteroids, and on the daily balances of phosphorus, potassium, and sodium have also been investigated.

The rationale for the glucagon-carbutamide experiments was provided by earlier work in this laboratory. Metabolic studies with fed diabetic patients^{8, 9} and with fasting experimental rats¹⁰ have shown that increased protein catabolism seems to be an integral part of the hyperglycemic-glycogenolytic response to fairly large doses of glucagon. Furthermore, studies of the rat have suggested that the increased protein catabolism induced by

* The work reported in this paper was supported by grants from Eli Lilly and Company, Indianapolis, Ind. and Chas. Pfizer & Co., Inc., Brooklyn, N. Y., and Research Grant A-611, from the National Institute of Arthritis and Metabolic Diseases, Public Health Service, Bethesda, Md.

† The results of such a study in one of the patients have been published in part in a preliminary report.⁹

glucagon is probably due to an enhanced hepatic gluconeogenesis from amino acids.

The second part of this paper will deal with the question of a possible hypoglycemic action of sulfonylureas in the severe, unstable diabetic receiving suboptimal doses of exogenous insulin. There is well-documented evidence showing that, in long-term studies, carbutamide and tolbutamide can lower the levels of glucose in blood and urine in depancreatized dogs receiving suboptimal doses of exogenous insulin,¹¹⁻¹⁴ but not in severe, unstable diabetes¹⁵⁻¹⁷ in humans similarly treated with suboptimal doses of exogenous insulin. With the introduction of more potent hypoglycemic agents such as chlorpropamide, it was considered worthwhile to reinvestigate this problem. The effects of tolbutamide and chlorpropamide on carbohydrate and protein metabolism have been determined for two patients with severe, unstable, long-standing diabetes, who were presumed to be critically lacking in endogenous insulin.

METHODS

Subjects

Four patients with diabetes mellitus served as subjects. One of the patients (R. E.) had the stable type of diabetes; the remaining three had the unstable type. The illness was of very recent onset in one of these unstable cases (A. F.); in the other two cases (E. R. and E. D.) it was of many years' duration. The subjects were classified as unstable or stable according to a criterion described in a previous paper.¹⁸ Throughout the period of study, which in each case extended several months, the patients lived quietly in the metabolism ward of Strong Memorial Hospital under routine but rigidly controlled conditions. Physical activity was usually restricted to being up and about in the ward as the patients wished.

Diets

Each patient received a weighed diet constant in chemical composition, the identical menu being served every day. The methods and rationale for calculating protein, fat, carbohydrate, total calories, and distribution of carbohydrate among the meals have been reported elsewhere.¹⁹

Insulin

Insulin was administered regularly to each patient as a single daily dose of either NPH or lente insulin every morning before breakfast. Initially, the patients were placed on approximately the same dose of insulin that they had been receiving before hospitalization. Subsequently, the dose was adjusted to achieve optimal regulation of blood and urinary sugar levels. In order to achieve more satisfactory regulation of the levels of sugar in blood and urine, patients A. F., E. R., and E. D. received also a small dose of crystalline insulin each morning before breakfast. Suboptimal regulation of blood and urinary sugar levels was achieved by appropriate reduction of the total daily dose of insulin to the point where the levels of blood and urinary sugar became

significantly higher than those obtained with optimal doses of insulin, but without the development of ketosis.

Analytical Procedures

All patients were weighed daily every morning before breakfast, and fasting blood sugar and 24-hour urinary excretion of sugar, nitrogen, phosphorus, potassium, and sodium were determined daily for each. A sample daily menu for each patient was analyzed for nitrogen, phosphorus, potassium, and sodium. In each case the average daily fecal excretion of nitrogen, phosphorus, potassium, and sodium was calculated from a pooled 5-day collection of feces. Carmine was used to mark the beginning and end of the period of collection. Daily balances for nitrogen, phosphorus, sodium, and potassium were obtained by subtracting the observed daily 24-hour urinary values and calculated average daily fecal values from measured amounts of nitrogen, phosphorus, sodium, and potassium in a sample diet. Fasting plasma, inorganic phosphorus, and 24-hour urinary excretion of reducing corticosteroids were determined daily in patients R. E. and A. F. In patient R. E. the fasting blood levels of carbutamide and capillary blood sugar were also measured daily.

Capillary samples of blood were drawn from the finger tip, usually in duplicate, within 1 or 2 min. of the venipuncture, using pipettes calibrated to contain 0.2 ml. The purpose of this procedure was to estimate the concentration of glucose in arterial blood (capillary blood was assumed to equal arterial blood in glucose concentration). The fasting levels of total plasma amino acids and chlorpropamide were determined daily in patient E. D.

Chemical Methods

Sugar in blood and urine was determined by the Shaffer-Hartman copper iodometric method,²⁰ using Somogyi's new copper reagent.²¹ Urines were analyzed for total nitrogen in duplicate by a modified micro-Kjeldahl method. Diets and stools were analyzed for total nitrogen in triplicate by the macro-Kjeldahl method, using the "wet" technique.²² Sodium and potassium in diets, stools, and urines were determined by means of a Baird flame photometer. Inorganic phosphorus in plasma, urine, diet, and stool was measured by the method of Fiske and SubbaRow.²³ Total reducing corticosteroids in urine were determined as follows: the urine was hydrolyzed for 48 hours at 47° C. with β -glucuronidase after adjustment to pH 4.5 and buffering. After the steroids had been extracted in chloroform a "neutral extract" of the latter²⁴ was prepared. Finally, the corticosteroids were estimated colorimetrically by a modification of the Mader and Buck method²⁵ using blue tetrazolium (BT).²⁴ Plasma amino acid nitrogen was measured by the method of Frame *et al.*²⁶ Serum carbutamide levels were measured by a modification of the method of Marshall and Bratton,²⁷ and serum chlorpropamide levels were determined by D. Iezzoni of Chas. Pfizer & Co., Inc., Brooklyn, N. Y.

Glucagon

Patients R. E. and A. F. were allowed to reach nitrogen equilibrium before the control data were obtained. After suitable periods (of several days)

duration) each patient received either 1 or 2 mg. of an aqueous solution of glucagon intramuscularly every 8 hours, 24 hours per day, for 6 days. One such course of glucagon was administered during the period of optimal regulation of blood and urinary sugar levels with exogenous insulin as described above, and one course was given during optimal regulation of sugar levels with carbutamide. The glucagon used in these studies was an approximately 50 per cent pure amorphous preparation.* It contained between 0.005 and 0.05 U. of insulin per milliliter of solution and bore the Lot No. 208-158B-214A.

Arylsulfonylureas

All of the sulfonylureas used in these studies were given by mouth daily, in divided doses. Patients R. E. and A. F. received 2.0 gm. and 2.5 gm. of carbutamide,* respectively, per day. Patient E. R. received 1.5 to 2.5 gm. of tolbutamide† per day, and patient E. D. was given 2 separate courses of chlorpropamide,‡ the first at 1.5 gm. per day and the second at 2.0 gm. daily. At least 2 or 3 days were allowed to elapse, after beginning or stopping treatment with the drug, before the metabolic data were obtained.

RESULTS

Effects of Carbutamide on the Responses of Various Metabolic Indices to Glucagon

The pertinent metabolic data obtained in patients R. E. and A. F. are shown in FIGURES 1 and 2 and in TABLES 1 and 2, respectively. All data are expressed as mean daily values ± 2 standard errors about the mean with the exception of the $\frac{A-V}{A}$ ratios in FIGURE 1. The latter values are expressed

as the mean daily values ± 1 standard error about the mean. This was done to avoid negative values about the mean, which would have occurred for some periods because of the marked variability in the daily observed values and because of the small size of the sample.

Blood and urinary sugar levels. Before comparing the responses of the blood and urinary sugar levels to glucagon when the patients were or were not receiving carbutamide, it may be well to point out that administration of carbutamide caused a striking fall in the levels of fasting blood sugar and in the excretion of glucose. This is evident from a comparison of the blood and urinary sugar levels in periods 1 and 5 with those of periods 6 and 8 in FIGURE 1, and of the levels in period 4 with those of periods 5, 7, and 8 in FIGURE 2. In patient R. E. the regulation of both the fasting blood sugar and the amount of sugar excreted in the urine was equal, if not superior, to that achieved by the administration of 36 U. of insulin daily, and in patient A. F. the regulation by carbutamide was as good as or better than the administration of 100 U. of insulin daily without carbutamide. In both cases the daily ingestion of 2.0 to 2.5 gm. of carbutamide resulted ultimately in fasting blood sugar levels close to or within the normal range, and the excretion of less than

* Supplied by Eli Lilly and Company, Indianapolis, Ind.

† Supplied by The Upjohn Company, Kalamazoo, Mich.

‡ Supplied by Chas. Pfizer & Co., Inc., Brooklyn, N. Y.

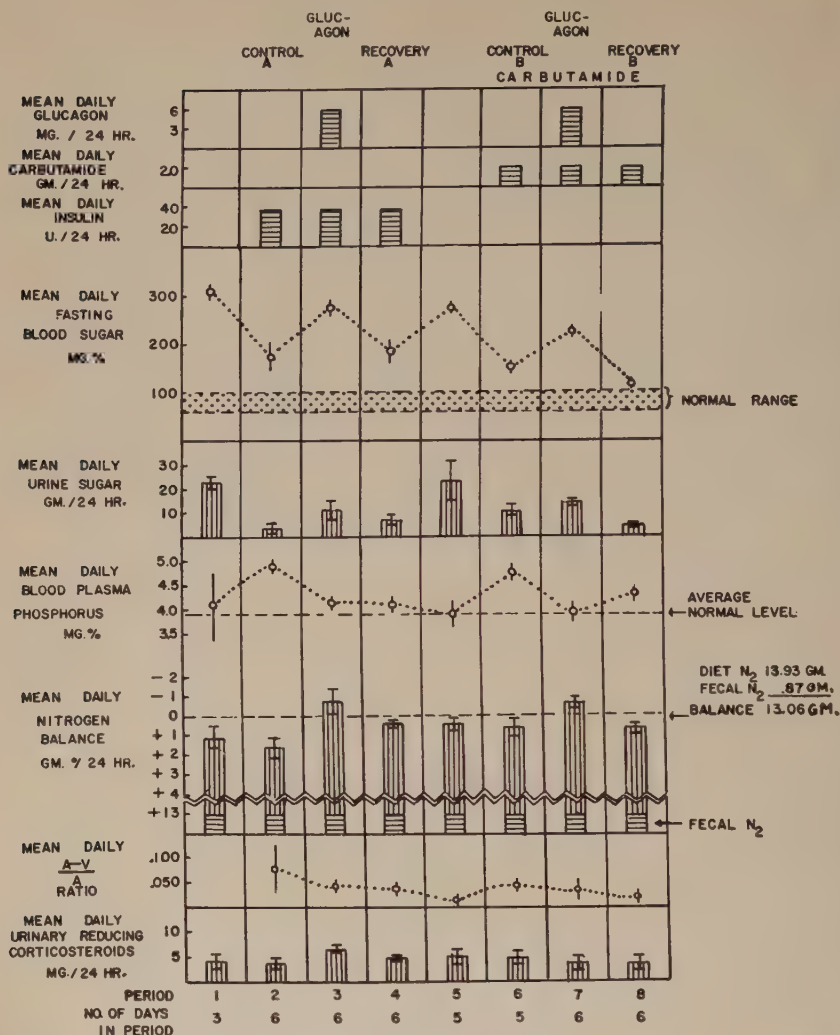


FIGURE 1. Effect of continued administration of carbutamide on the various metabolic responses to intramuscular injections of glucagon every 8 hours for 6-day periods. All data are expressed as mean daily values ± 2 S.E. about the mean, except for the $\frac{A-V}{A}$ ratio, which is expressed as the mean daily fasting ratio ± 1 S.E. about the mean. For further explanation see text. Patient R. E., female, 64 years old, stable diabetes, long duration.

2.0 gm. of glucose in the urine every 24 hours. The mean daily level of blood carbutamide was 7.7 mg. per cent in patient R. E. and 7.14 mg. per cent in patient A. F. It should be noted that there was greater consistency in fasting blood sugar levels from day to day when the patients were receiving carbutamide than when they were receiving insulin only, as shown by the

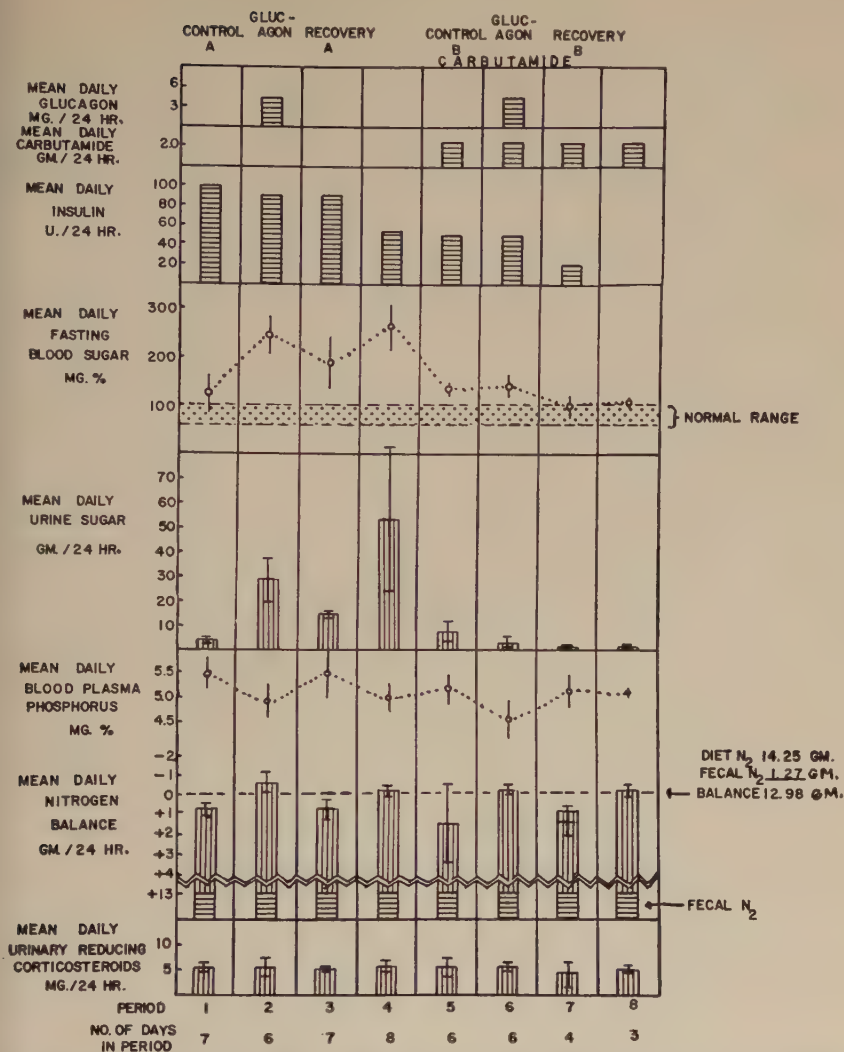


FIGURE 2. Effect of continued administration of carbutamide on the various metabolic responses to intramuscular injections of glucagon every 8 hours for 6-day periods. All data are expressed as mean daily values ± 2 S.E. about the mean. Adapted from Izzo and Roncone⁹ (by permission of *Diabetes, The Journal of The American Diabetes Association*). Patient A. F., female, 19 years old, unstable diabetes, short duration.

fact that a smaller standard error was obtained with carbutamide than with insulin. Moreover, the need for insulin in both patients is evident from the levels of hyperglycemia and the glycosuria that resulted when insulin was omitted (periods 1 and 5, FIGURE 1) or reduced to suboptimal levels (period 4, FIGURE 2).

Intramuscular injections of glucagon administered to both patients during

TABLE 1
PHOSPHORUS, SODIUM, AND POTASSIUM BALANCE DATA OF PATIENT R. E.: FEMALE, 64 YEARS OLD, STABLE DIABETES OF LONG DURATION*

	Period							
	1	2	3	4	5	6	7	8
No. of days.....	3	6	6	5	5	6	6	5
Carbutamide, gm./24 hours.....		6	36			2.0	2.0	2.0
Glucagon, mg./24 hours.....		36		36			6	
Insulin, U./24 hours.....								
Urine inorganic phosphorus, gm./24 hours.....	.628† ± .058	.568 ± .110	.680 ± .066	.597 ± .124	.396 ± .134	.510 ± .216	.729 ± .028	.652 ± .058
Urine sodium, mEq./24 hours.....	63.7† ± 12.0	40.2 ± 5.0	54.8 ± 13.4	43.2 ± 6.8	61.0 ± 5.4	63.4 ± 9.2	61.8 ± 5.6	51.4 ± 8.0
Urine potassium, mEq./24 hours.....	74.4† ± 7.2	77.6 ± 8.6	87.2 ± 13.8	84.2 ± 3.6	87.4 ± 7.4	80.5 ± 7.0	79.7 ± 5.4	87.5 ± 2.4

* For further details see text and FIGURE 1. All urinary values are expressed as the mean ± 2 S.E. about the mean.

† To obtain the daily balance, subtract the urinary value from the average daily intake; urinary values less than the average daily intake indicate positive balance, and urinary values greater than the average daily intake indicate negative balance:

Sample daily diet	Phosphorus, gm.	Potassium, mEq.	Sodium, mEq.
Average daily fecal excretion	1.606	92.04	58.15
	— .686	— 4.06	— 1.93
Average daily intake	.920	87.98	56.22

TABLE 2
PHOSPHORUS, SODIUM, AND POTASSIUM BALANCE DATA OF PATIENT A. F.; FEMALE, 19 YEARS OLD, UNSTABLE DIABETES OF SHORT DURATION*

No. of days	Period							
	1	2	3	4	5	6	7	8
Carbutamide, gm./24 hours.....								
Glucagon, mg./24 hours.....								
Insulin, U./24 hours.....								
Urine inorganic phosphorus, gm./24 hours.....	100	3-6 90	90	54	2.5 50	2.5 3-6 50	2.5 35	2.5
Urine sodium, mEq./24 hours.....	.950† ± .136	.986 ± .104	.890 ± .174	1.061 ± .132	.945 ± .114	1.008 ± .074	.912 ± .176	.921 ± .138
Urine potassium, mEq./24 hours.....	64.5† ± 21.4	63.3 ± 28.0	62.0 ± 29.0	65.5 ± 27.0	64.6 ± 5.8	64.2 ± 16.8	63.5 ± 19.8	66.9 ± 38.6
Urine potassium, mEq./24 hours.....	62.7† ± 3.0	59.1 ± 5.2	61.0 ± 5.6	68.8 ± 8.4	63.0 ± 4.2	75.3 ± 6.2	69.9 ± 18.8	79.7 ± 1.2

* For further details see text and FIGURE 2. All urinary values are expressed as the mean ± 2 S.E. about the mean.

† To obtain the daily balance, subtract the urinary value from the average daily intake; urinary values less than the average daily intake indicate positive balance, and urinary values greater than the average daily intake indicate negative balance:

Sample daily diet	Phosphorus, gm.	Potassium, mEq.	Sodium, mEq.
Average daily fecal excretion	1.377	82.38	70.46
	— .540	— 18.31	— 6.39
Average daily intake	.837	64.07	64.07

the time of optimal regulation with exogenous insulin resulted in sharp increases in fasting blood sugar levels and 24-hour excretion of sugar (periods 2 and 3, FIGURE 1, and periods 1 and 2, FIGURE 2). The mean increase in daily fasting blood sugar above control levels was 101 mg. per cent in patient R. E. and 119 mg. per cent in patient A. F. The mean increases in daily urinary sugar levels were 7.31 gm. and 24.47 gm., respectively. With discontinuation of glucagon, the levels of blood and urinary sugar promptly moved toward the control levels (period 4, FIGURE 1 and period 3, FIGURE 2). Intramuscular administration of glucagon to patient R. E. during the time she was receiving carbutamide resulted in a mean increase in fasting blood sugar level, which was only slightly less than that caused by similar injections of glucagon during the time of optimal insulin regulation (periods 7 and 8, FIGURE 1). The mean rise in 24-hour excretion of sugar was 3.53 gm. It should be noted that the rise in the levels of fasting blood sugar induced by glucagon when patient R. E. was receiving either insulin or carbutamide was not associated with any increase in peripheral glucose utilization, as measured by the $\frac{A-V}{A}$ ratio. In sharp contrast to the results obtained in patient

R. E., the administration of glucagon to patient A. F. at a time when she was receiving carbutamide with reduced doses of insulin produced no rise in levels of blood or urinary sugar (periods 5, 6, and 7, FIGURE 2).

Nitrogen balances. Patients R. E. and A. F. were both in positive nitrogen balance when they were receiving optimal doses of exogenous insulin (period 2, FIGURE 1 and period 1, FIGURE 2). In addition to increasing the levels of glucose in the blood and urine, glucagon also caused a significant rise in the excretion of nitrogen in the urine, with a reversal of nitrogen balance from positive to slightly negative (period 3, FIGURE 1 and period 2, FIGURE 2). The mean daily increase in the excretion of nitrogen during the period of glucagon administration was 2.39 gm. higher than the control levels in patient 1, and 1.40 gm. higher than the control levels in patient 2. With discontinuance of glucagon, the excretion of nitrogen promptly decreased and the nitrogen balances again became positive (period 4, FIGURE 1 and period 3, FIGURE 2). It should be noted that patient R. E. was in positive nitrogen balance during periods 1 and 5, FIGURE 1 when insulin was omitted, even though the levels of hyperglycemia and glycosuria were similar to or even higher than the levels produced by glucagon and insulin. Glucagon also caused a greater excretion of nitrogen in patient A. F. than did reduction of insulin to suboptimal levels, although the hyperglycemia and glycosuria were greater during the period of suboptimal insulin administration than during the period of administration of the optimal amount of insulin together with glucagon.

The administration of glucagon in the presence of carbutamide also resulted in a significant increase in the excretion of nitrogen in the urine in patients R. E. and A. F. The rises were roughly of the same order of magnitude as the rise in nitrogen excretion that occurred when the patients were given glucagon while receiving optimal doses of insulin. Nitrogen balances

reverted from positive to slightly negative in patient R. E., and to equilibrium in the case of patient A. F. (periods 6 and 7, FIGURE 1, and periods 5 and 6, FIGURE 2). The increase in the mean daily nitrogen excretion induced by administration of glucagon was 1.29 gm. in patient R. E. and 1.67 gm. in patient A. F. As in the glucagon-insulin studies, nitrogen excretion promptly decreased, and the nitrogen balances again became positive when the injections of glucagon were stopped (period 8, FIGURE 1, and period 7, FIGURE 2). It is of interest to note that carbutamide did not diminish the nitrogen response to glucagon in patient A. F., even though it virtually abolished the hyperglycemic-glycosuric response to glucagon. Nitrogen retention was greater in patient A. F. when she was receiving carbutamide and reduced doses of insulin than when carbutamide alone was given, although the regulation of blood and urinary sugar levels in both periods 7 and 8, FIGURE 2, was similar. However, because periods 7 and 8 were of short duration, little significance can be attached to these differences in nitrogen excretion.

Plasma inorganic phosphorus. Before discussing the effect of carbutamide on the response of the level of plasma inorganic phosphorus to the administration of glucagon, it is pertinent to point out the occurrence of an inverse relationship between the level of fasting blood sugar and the level of fasting plasma inorganic phosphorus in both patients R. E. and A. F. This is shown in patient R. E. by the significant rise in mean daily level of plasma inorganic phosphorus from 4.11 mg. per cent to 4.88 mg. per cent as the fasting blood sugar level decreased from 309 mg. per cent to 173 mg. per cent (periods 1 and 2, FIGURE 1). The phosphorus-glucose relationship is also seen in patient A. F. The mean daily levels of fasting blood sugar and fasting plasma inorganic phosphorus during the periods of optimal insulin therapy (period 1, FIGURE 2) were 125 mg. and 5.46 mg. per cent, respectively; the mean daily levels of fasting blood sugar and plasma inorganic phosphorus during the period of suboptimal insulin therapy (period 4, FIGURE 2) were 263 mg. per cent and 4.98 mg. per cent, respectively. In fact, a close inspection of the mean daily levels of fasting blood sugar and plasma inorganic phosphorus for all periods in both FIGURES 1 and 2 suggest, with a few exceptions described below, that there is an inverse relationship between these two variables irrespective of the type of treatment. The glucose-phosphorus relationships just described are similar to those reported previously in diabetes.^{9, 28} They are of interest since they are the reverse of the well-known short-term relationship between glucose and phosphorus, which has been observed in nondiabetics following the administration of glucose or insulin.

The sharp rises in levels of fasting blood sugar that occurred in patients R. E. and A. F. when glucagon was administered while the patients were receiving optimal doses of insulin were associated with simultaneous decreases in levels of plasma inorganic phosphorus (periods 2 and 3, FIGURE 1, and periods 1 and 2, FIGURE 2). The mean decreases in levels of plasma inorganic phosphorus in patients R. E. and A. F. were 0.76 and 0.56 mg. per cent, respectively. Discontinuation of glucagon resulted in a prompt return of plasma inorganic phosphorus to control levels (period 4, FIGURE 1, and

period 3, FIGURE 2). The administration of carbutamide did not alter the hypophosphatemic response to injections of glucagon in either patient (period 7, FIGURE 1, and period 6, FIGURE 2), even though, in one of these patients (A. F.), carbutamide virtually abolished the hyperglycemic response to glucagon administration. The decreases during glucagon administration in the mean daily levels of serum inorganic phosphorus below the control levels in patients R. E. and A. F. were 1.02 mg. and 0.63 mg. per cent, respectively. As was the case with glucagon administration during periods of optimal insulin therapy, discontinuation of glucagon resulted in a prompt return of serum inorganic phosphorus toward control levels. It should be noted that in patient R. E. the fall in serum inorganic phosphorus induced by glucagon in periods 3 and 7 was not associated with any significant increase in peripheral glucose utilization as measured by changes in the $\frac{A-V}{A}$ ratio.

Urinary reducing corticosteroids. The mean daily urinary excretion of reducing corticosteroids was within the normal range in both cases. Glucagon administration produced a slight but significant rise in the mean daily excretion of urinary reducing corticosteroids in the case of patient R. E. when she was being treated with optimal doses of insulin (periods 2 and 3, FIGURE 1), but this did not occur when glucagon was again administered while the patient was receiving carbutamide instead of insulin (periods 6 and 7, FIGURE 1). Neither glucagon nor carbutamide had any effect on the level of excretion of urinary reducing corticosteroids in patient A. F.

Phosphorus, potassium, and sodium balances. Slight increases in the mean daily urinary excretion of phosphorus were observed when both patients R. E. and A. F. were given glucagon either while receiving optimal doses of insulin or carbutamide in periods 2, 3, 4, 6, 7, and 8 in Table 1, and in periods 1, 2, 3, 5, 6, and 7 in Table 2. The increases in inorganic phosphorus excretion induced by glucagon were highly significant ($p < 0.01$) in the case of patient R. E. (only when carbutamide was given), but were not statistically significant ($p > 0.05$) in the case of patient A. F. Although the daily balances for sodium and potassium varied markedly from day to day and period to period in both patients, no relationship could be established between the changes in balances of sodium and potassium and the administration of glucagon and carbutamide either singly or together.

Metabolic Effects of Sulfonylureas in Patients with Severe, Long-Standing, Unstable Diabetes of the Juvenile Type

Tolbutamide. The oral administration of 2.0 gm. of tolbutamide daily to patient E. R. (FIGURE 3) for 12 days did not lower the levels of blood and urinary sugar (periods 1 and 2). In fact, the glycosuria tended to increase while the patient was receiving tolbutamide. It should be pointed out that this patient was receiving only sufficient insulin daily to maintain the fasting blood sugar at slightly above the normal range, and the mean 24-hour excretion of glucose in the urine at approximately 25 gm. Ketonuria was

uniformly absent in all urine specimens throughout the day. Administration of carbutamide also failed to affect the levels of plasma inorganic phosphorus or mean daily nitrogen balances. It should be noted that with the spontaneous fall in levels of blood and urinary sugar (period 3) the nitrogen

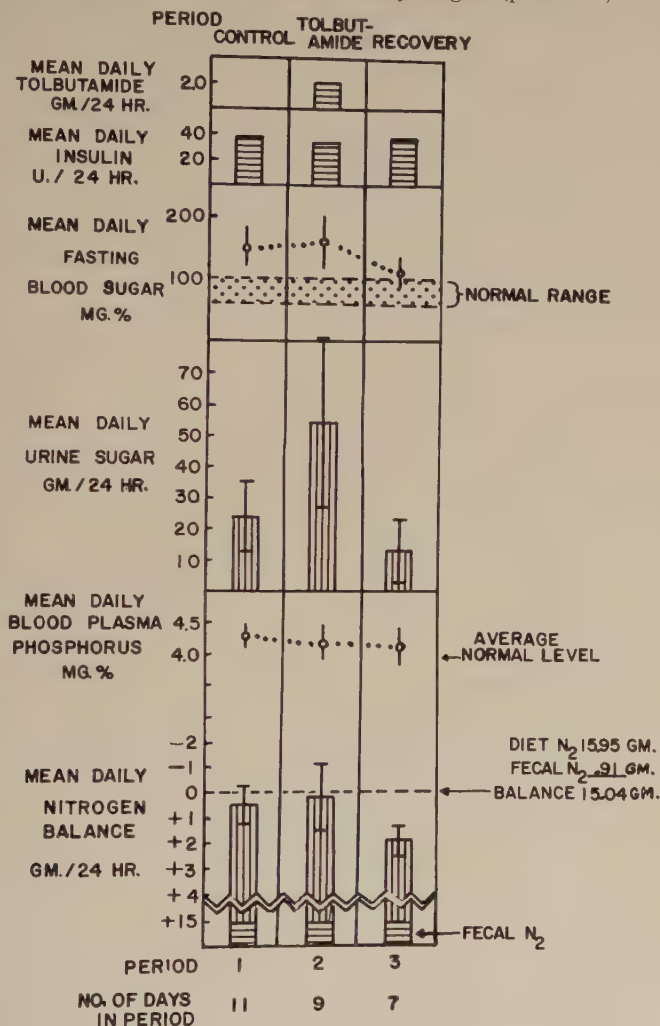


FIGURE 3. Effect of continued administration of tolbutamide in a severe, unstable diabetic maintained on suboptimal daily doses of insulin. All data are expressed as mean daily values ± 2 S.E. about the mean. Patient E. R., male, 50 years old, unstable diabetes, long duration.

balance became more positive even though the daily dose of insulin was maintained constant throughout the experiment.

The administration of tolbutamide had no significant effects on the urinary excretion of phosphorus, sodium, or potassium (TABLE 3).

Chlorpropamide. This compound was administered orally to patient E. D. during 2 different periods separated by an adequate recovery interval (FIGURE 4). On 24 U. of insulin daily (20 U. of lente insulin plus 4 U. of crystalline insulin), the mean daily fasting blood sugar level during the 11-day control period was 247 mg. per cent, and the mean daily excretion of sugar in the urine was 50.2 gm. (period 1). With the administration of 1.0 to 1.5 gm. of chlorpropamide per day in addition to the 24 U. of insulin per day for 16 days (period 2), the mean fasting daily blood sugar level was 188 mg. per

TABLE 3
PHOSPHORUS, SODIUM, AND POTASSIUM BALANCE DATA OF PATIENT E. R.: MALE,
50 YEARS OLD, UNSTABLE DIABETES OF LONG DURATION*

	Period		
	1	2	3
	10	10	6
No. of days.....			
Tolbutamide, gm./24 hours.....		2.0	
Insulin, U./24 hours.....	36	34	36
Urine inorganic phosphorus, gm./24 hours.....	.796 \pm .048	.794 \pm .090	.756 \pm .106
Urine sodium, mEq./24 hours.....	80.4 \pm 13.6	96.5 \pm 16.4	76.6 \pm 16.4
Urine potassium, mEq./24 hours..	89.4 \pm 27.2	88.4 \pm 33.8	80.4 \pm 21.2

* For further details see text and FIGURE 3. All urinary values are expressed as the mean \pm 2 S.E. about the mean.

† To obtain the daily balance, subtract the urinary value from the average daily intake; urinary values less than the average daily intake indicate positive balance, and urinary values greater than the average daily intake indicate negative balance:

	Phosphorus, gm.	Potassium, mEq.	Sodium, mEq.
Sample daily diet	1.917	98.52	102.62
Average daily fecal excretion	-1.054	- 8.43	- 1.78
Average daily intake	.863	90.09	100.84

cent, and the mean daily excretion of sugar was 26.7 gm. The decrease in level of fasting blood sugar was just significant at the 5 per cent level, while the decrease in the amount of glucose excreted in 24 hours was significant at less than the 5 per cent level. With the discontinuation of chlorpropamide, both the mean daily fasting blood sugar and the mean 24-hour excretion of sugar returned toward control values (period 3). The mean daily fasting blood sugar level in period 3 was 234 mg. per cent and the mean daily excretion of glucose 41.5 gm. After a 10-day recovery interval chlorpropamide was administered again (period 4). On this occasion the daily dose of chlorpropamide was increased to 2.0 gm. per day, while the daily dose of lente insulin was decreased to 16 U. per day, with the 4 U. of crystalline insulin omitted. There was no change in the 24-hour excretion of glucose

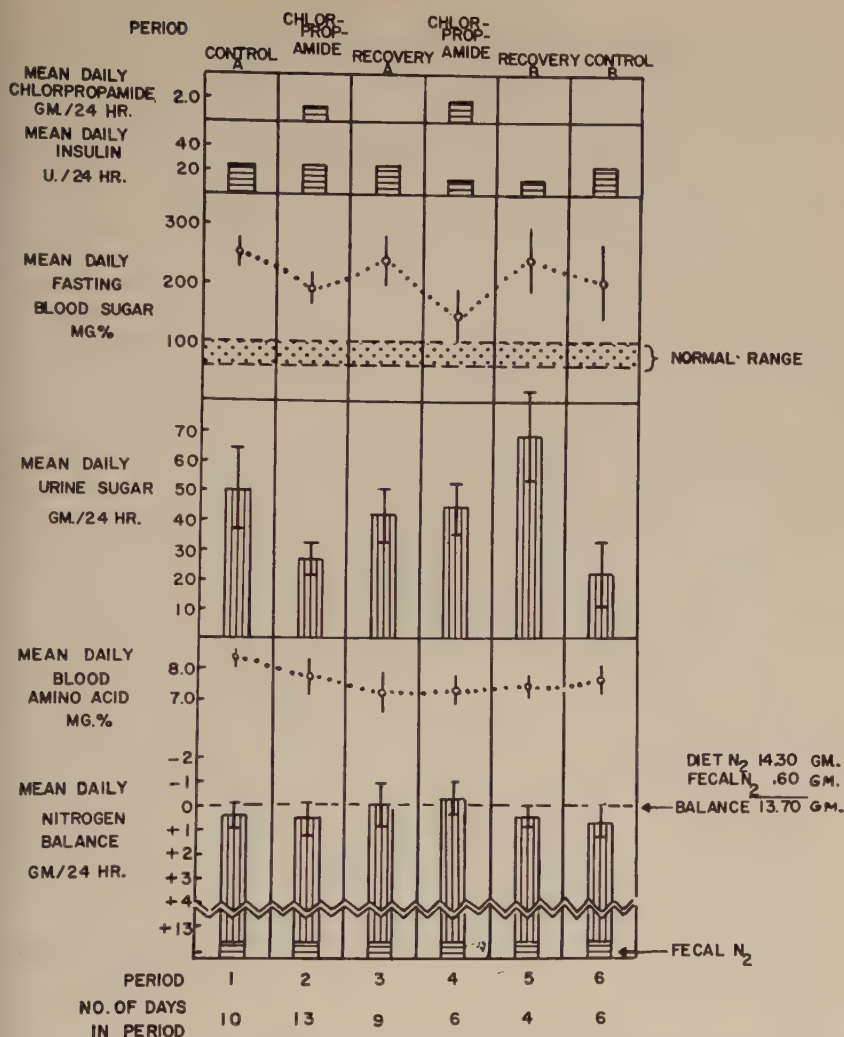


FIGURE 4. Effect of continued administration of chlorpropamide in a severe, unstable diabetic maintained on suboptimal daily doses of insulin. All data are expressed as mean daily values ± 2 S.E. about the mean. Patient E. D., female, 31 years old, unstable diabetes, long duration.

in the urine (43.9 gm. in period 4 as compared to 41.5 gm. in period 3), but the fasting blood sugar fell from a mean daily value of 234 mg. per cent in period 3 to 142 mg. per cent in period 4. The decrease of 92 mg. in the mean daily fasting blood sugar level was significant beyond the 5 per cent level. When the chlorpropamide was discontinued but the daily dose of insulin maintained at 16 U. of lente insulin per day, the fasting blood sugar level moved toward but did not exceed the control levels, whereas the mean daily

TABLE 4
PHOSPHORUS, SODIUM, AND POTASSIUM BALANCE DATA OF PATIENT E. D.: FEMALE,
31 YEARS OLD, UNSTABLE DIABETES OF LONG DURATION*

	Period					
	1	2	3	4	5	6
No. of days.....	11	14	8	6	4	6
Chlorpropamide, gm./24 hours.....		1.5		2.0		
Insulin, U./24 hours..	24	24	24	16	16	24
Urine inorganic phos- phorus, gm./24 hours.....	.807† ± .068	.723 ± .066	.789 ± .044	.809 ± .136	.740 ± .066	.771 ± .108
Urine sodium, mEq./24 hours....	68.3† ± 6.8	65.9 ± 11.4	47.4 ± 8.4	99.2 ± 32.2	45.6 ± 16.0	56.5 ± 18.2
Urine potassium, mEq./24 hours....	99.4† ± 10.8	75.6 ± 10.4	75.6 ± 5.8	88.5 ± 8.2	94.0 ± 11.0	92.5 ± 9.4

* For further details see text and FIGURE 4. All urinary values are expressed as the mean ± 2 S.E. about the mean.

† To obtain the daily balance, subtract the urinary value from the average daily intake; urinary values less than the average daily intake indicate positive balance, and urinary values greater than the average daily intake indicate negative balance:

	Phosphorus, gm.	Potassium, mEq.	Sodium, mEq.
Sample daily diet	1.412	130.94	79.40
Average daily fecal excretion	— .619	— 1.37	— .92
Average daily totals	.793	129.57	78.48

excretion of sugar in the urine (68.0 gm. per day) exceeded that of any previous period. Increasing the insulin to the previous level of 20 U. lente insulin and 4 U. of crystalline insulin per day resulted in a slight but insignificant decrease in the mean daily level of fasting blood sugar, but a sharp fall in the mean daily level of urinary sugar to 21.8 gm. for 24 hours.

Chlorpropamide appeared to have no effect on the level of plasma amino nitrogen or daily nitrogen balance, nor was the administration of chlorpropamide associated with any consistent change in the daily balances of phosphorus, potassium, or sodium (TABLE 4). The mean daily level of chlorpropamide in the plasma on a daily dose of 1.0 to 1.5 gm. of the drug was 15.2 mg. per cent.

DISCUSSION

As stated in the introduction to this paper, previous studies have shown that repeated daily intramuscular injections of glucagon in diabetic subjects result in an increased catabolism of protein, which is evidenced by an increased excretion of nitrogen and a reversal of nitrogen balance from positive to negative. The experimental studies in the rat have suggested that this effect of glucagon on protein catabolism is probably related largely to an increased hepatic gluconeogenesis from amino acids. In the present studies, although administration of carbutamide to patients R. E. and A. F. resulted in a striking hypoglycemic response, it failed to block or suppress the increased excretion of nitrogen and phosphorus induced by glucagon. This would

argue against the possibility that the reduction in hepatic glucose output associated with administration of sulfonylureas is due in any significant degree to selective interference with hepatic gluconeogenesis from amino acids. Furthermore, as these and other studies^{9, 29} have shown, none of the sulfonylurea compounds tested thus far has shown any direct effects on protein metabolism as measured by changes in daily balances of nitrogen, phosphorus, and potassium, or by changes in levels of plasma amino acids. Adrenocortical function as measured by changes in levels of urinary reducing corticosteroids was not depressed by administration of sulfonylureas. It seems unlikely that sulfonylurea compounds decrease hepatic glucose output by interfering with protein mobilization or breakdown.

It is of interest that in one of the two patients studied (A. F.), carbutamide failed to block the protein catabolic action of glucagon, even though the hyperglycemic action appeared to be virtually abolished. We have no explanation at the present time for this dissociation of glucagon effects by carbutamide. Tolbutamide has been found to reduce the rate of glycogenolysis in liver slices stimulated by epinephrine.^{3, 30} Nevertheless, attempts to demonstrate inhibitory action of either carbutamide or tolbutamide on the acute hyperglycemia induced by intravenous injection of glucagon or subcutaneous injections of epinephrine have been largely unsuccessful.^{7, 29, 31} The present studies show that, in some diabetics, carbutamide may block the increase in levels of blood and urinary sugar induced by repeated daily intramuscular injections of glucagon. However, the known ability of sulfonylureas to block or interfere with glycogenolysis *in vitro*,^{3, 30} does not seem to be essential for their hypoglycemic action, since our studies show that administration of carbutamide was associated with hypoglycemia irrespective of whether the hyperglycemic response to glucagon was suppressed.

The negative correlation between levels of fasting blood sugar and levels of plasma inorganic phosphorus observed in patients E. R. and A. F. has been described.²⁸ In general, low levels of blood sugar tend to be associated with high levels of plasma inorganic phosphorus, and vice versa, in the diabetic. The findings are of interest since they are the reverse of the well-known short-term changes in plasma phosphorus associated with the intravenous administration of insulin to nondiabetics.²⁸

Glucagon injection induced a fall in plasma phosphate in both patients, not only when they received insulin, but also when they received carbutamide. The fact that injections of glucagon resulted in a sharp lowering of plasma phosphate even when the hyperglycemic response appeared to be suppressed by carbutamide suggests that the lowering of phosphate is directly related in some unknown fashion to the administration of glucagon rather than to the hyperglycemia per se. In this respect, one may speculate that the inverse relationship between changes in fasting blood sugar and plasma phosphate in diabetes may be the result of changes in the level of secretion or the activity of endogenous glucagon. It is pertinent to point out that the rise in blood sugar and the fall in plasma phosphorus induced by the administration of glucagon were not associated with any increase in peripheral glucose utiliza-

tion as measured by changes in the *A-V* glucose difference. This suggests that the phosphorus was not entering into the glycolytic cycle of peripheral tissues. It is possible that, under the conditions described, the fall of inorganic phosphorus may represent entry of phosphorus in the glycogenic cycle in the liver, and that glucagon may be involved in the regulation of these processes. A different type of study would be needed to establish or disprove the validity of this hypothesis.

The failure of tolbutamide to lower blood or urinary sugar levels in patient E. R. is in agreement with the results of other investigators who have been unable to demonstrate a hypoglycemic action of tolbutamide or carbutamide in severe, unstable diabetics.¹⁵⁻¹⁷ For this reason the lowering of blood and urinary sugar levels in patient E. D., which appeared to be associated with the administration of chlorpropamide, is of interest. The results suggest the possibility that some types of sulfonylurea compounds may have a hypoglycemic action in severe, unstable diabetics when a certain minimal amount of insulin is administered. Since patient E. D. was found to be critically lacking in endogenous insulin, it would be difficult to explain the hypoglycemic response to chlorpropamide on the basis of stimulation of the insulogenic function of the pancreas. It is reasonable to suspect that a different type of mechanism may be involved. Chlorpropamide did not affect the daily nitrogen balances in this patient. However, we do not consider this as strong evidence against the possibility of an "insulinlike" action of the sulfonylureas, because the patient remained in nitrogen equilibrium while she was being suboptimally regulated with insulin without chlorpropamide.

The findings that have been discussed are not all readily explainable on the basis of a single locus of action of sulfonylureas. As pointed out, the probable hypoglycemic action of chlorpropamide in patient E. D., who was presumed to be critically deficient in endogenous insulin, and the inhibition of the hyperglycemic response to glucagon by carbutamide in patient A. F. are difficult to explain on the basis of stimulation of the beta cells by the sulfonylureas to produce more insulin. Nevertheless, the results of this study are not inconsistent with the hypothesis that the main action of sulfonylurea compounds is in the pancreatic islets. This does not necessarily mean, however, that the sulfonylureas stimulate the beta cells to release or secrete more insulin, as some investigators have suggested.³¹⁻³⁴ It seems possible, for example, that the sulfonylureas might act by decreasing the ratio of glucagon to insulin through an inhibiting action on glucagon-producing cells. However, a hepatic effect of glucagon cannot yet be excluded on the basis of the work reported above.

According to available evidence, the hypoglycemic action of sulfonylureas is associated with a concomitant reduction in hepatic glucose output,¹⁻⁵ which probably occurs without a concomitant increase in the peripheral utilization of glucose.³⁵⁻³⁷ In view of the now well-established action of insulin in increasing the permeability of peripheral tissues for sugar, the findings described have been interpreted by some investigators as evidence that the hypoglycemic action of sulfonylureas is not insulinlike and, therefore, not the result of stimulation of beta cells by sulfonylureas.^{35, 37} This conclusion

seems reasonable to us, but some investigators⁵ hold that the sulfonylureas stimulate the beta cells to release or secrete small quantities of insulin directly into the portal venous system. This small amount of released endogenous insulin is then supposed to act directly on the liver, causing a reduction in hepatic glucose output. The low levels of insulin in question are not thought to act beyond the liver and, hence, are not thought to be sufficient to increase peripheral glucose uptake as measured by available techniques. It should be pointed out, however, that a direct action of insulin on the liver has not yet been unequivocally established.³⁸ It appears to us that a more plausible explanation can be suggested, as has been done above, for the well-established observation that sulfonylurea compounds reduce hepatic glucose output without at the same time increasing peripheral glucose utilization. Glucagon is known to have a well-defined action on the liver, but as yet no action has been established for peripheral tissues.

One of the chief arguments raised to refute the possible action of sulfonylurea compounds on glucagon production is that the compounds have no hypoglycemic effect in alloxan-diabetic animals³⁴ in which, presumably, the alpha cells are intact. This may not be a sufficient basis for excluding the hypothesis of an alteration in the glucagon-insulin ratio as a mechanism of action of sulfonylureas, however; it may well be that some insulin must be present in order that the effects of a diminution in glucagon may be observed. This idea seems rational because, in the absence of insulin, the utilization of glucose by the periphery is too small to cause a lowering of blood sugar sufficient to expose the failure of glucagon to act.

SUMMARY AND CONCLUSIONS

The metabolic effects of prolonged administration of carbutamide, tolbutamide, and chlorpropamide have been investigated in four selected diabetic subjects under controlled conditions in the metabolism ward of the Strong Memorial Hospital.

Except for an effect on blood and urinary sugar levels, none of the compounds that were studied appeared to have any direct or consistent effect on the following metabolic parameters: (1) daily balances of phosphorus, potassium, and sodium; (2) daily balances of nitrogen; (3) daily levels of fasting plasma α -amino acid nitrogen; and (4) daily urinary excretion of corticosteroids.

The effects of sulfonylureas on hepatic gluconeogenesis and glycogenolysis were investigated in two diabetic subjects who showed a striking hypoglycemic response to carbutamide. This was done by studying the effect of prolonged administration of carbutamide on the ability of repeated daily intramuscular injections of glucagon to increase the urinary excretion of nitrogen as well as to increase the levels of glucose in blood and urine. The influence of carbutamide on the ability of glucagon to depress the level of serum inorganic phosphorus was also investigated. Previous studies from this laboratory had demonstrated that the increased protein catabolism that is an integral part of the hyperglycemic-glycogenolytic action of glucagon is related to an enhanced hepatic gluconeogenesis from protein.

Carbutamide markedly diminished the hyperglycemic and glycosuric response to glucagon in one patient, but had no effect on this response in the other patient, even though the drug had produced markedly decreased levels of blood and urinary sugar in both patients. However, in neither patient was there any influence of carbutamide on the increased protein catabolism or the depression of serum phosphorus caused by the administration of glucagon. These results suggest that the well-established action of sulfonylureas in reducing hepatic glucose output is not dependent upon either an inhibition of the hyperglycemic-glycogenolytic action of glucagon or a selective interference with hepatic gluconeogenesis from protein.

The present studies confirm the previous reports from this laboratory of the occurrence of an inverse relationship between the levels of fasting blood glucose and serum inorganic phosphorus in the diabetic. The possible role of glucagon in respect to this relationship is analyzed.

Also investigated was the ability of a prolonged administration of tolbutamide and chlorpropamide to reduce the levels of blood and urinary glucose in two cases of severe, unstable diabetes where there was presumed to be a critical deficiency of insulin. Both patients were maintained on sub-optimal doses of insulin. One patient was given tolbutamide and the other, chlorpropamide. Tolbutamide was ineffective in reducing the levels of glucose in blood or urine; chlorpropamide administration was associated with significant lowering of levels of blood and urinary sugar. The findings suggest that the hypoglycemic action of sulfonylureas may not be entirely dependent upon remaining islet-cell reserve and that the capacity may vary with different compounds.

The metabolic effects of sulfonylureas that have been described are inconsistent with the hypothesis of a single mechanism of action, such as a stimulation of the beta cells to secrete or release insulin. However, the results do not preclude the hypothesis that the central locus of action of sulfonylureas is pancreatic islet tissue. It is possible that the action of sulfonylureas in reducing hepatic glucose output, apparently without increasing peripheral glucose utilization, is due to a reduction in the ratio of secreted glucagon to secreted insulin, rather than to stimulation of the beta cells. This possibility is analyzed.

ACKNOWLEDGMENT

I express my appreciation of the assistance of Angela M. Roncone in the preparation of this paper.

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METABOLIC STUDIES OF CHLORPROPAMIDE IN NORMAL MEN AND IN DIABETIC SUBJECTS*

Raymond E. Knauff,† Stefan S. Fajans, Eduardo Ramirez,‡
Jerome W. Conn

Metabolism Research Unit, Division of Endocrinology and Metabolism, Department of Internal Medicine, University of Michigan Medical School, Ann Arbor, Mich.

We have conducted short-term and prolonged studies in normal men and in diabetic subjects to compare the physiological properties of chlorpropamide and tolbutamide. The primary objectives of the investigation were: first, to correlate the hypoglycemic effect with the serum sulfonylurea level for each compound; and, second, to compare the rates of disappearance of the two drugs from the blood. In addition, we performed metabolic balance studies in two normal men during the administration of chlorpropamide.

Methods

Serum sulfonylurea determinations. Serum concentrations of both chlorpropamide and tolbutamide were determined by the same analytical method. This method, developed by Toolan and Wagner for chlorpropamide, and described elsewhere in this monograph, was found to serve equally well for the determination of tolbutamide.

The performance of the method in this laboratory is summarized in TABLE 1.

TABLE 1
PERFORMANCE OF THE ANALYTIC METHOD IN THE DETERMINATION OF SERUM
CHLORPROPAMIDE AND TOLBUTAMIDE CONCENTRATIONS

Recovery of the sulfonylureas from serum:

Chlorpropamide	97.0 \pm 1.2%
Tolbutamide	99.0 \pm 0.8%

Features of the method:

Reproducibility (standards)

\pm 0.25 mg./100 ml. sulfonylurea

Precision (intraset serum replicates)

\pm 0.13 mg./100 ml. sulfonylurea

Accuracy (interset serum duplicates)

\pm 0.23 mg./100 ml. sulfonylurea

Values represent mean and/or standard deviation. The indicated values were derived as follows: recovery, 6 prepared serum samples each for chlorpropamide and tolbutamide between the concentrations of 0 to 24 mg./100 ml.; reproducibility, variation of the mid-level standard in 35 sets; precision, variation among 9 intraset serum replicates; accuracy, variation among 30 serum samples, as 15 duplicate pairs with concentrations of 1 to 35 mg./100 ml., scattered among 13 sets.

The recovery of both sulfonylureas added to serum was essentially complete.

* The work reported in this paper was supported in part by Chas. Pfizer & Co., Inc., Brooklyn, N. Y., and in part by Grant A-888 C3 from the National Institute of Arthritis and Metabolic Diseases, Public Health Service, Bethesda, Md.

† Investigator, the Howard Hughes Medical Institute, Miami Beach, Fla.

‡ Foreign Educational Fellow, Eli Lilly and Company, Indianapolis, Ind.

The method, which showed excellent reproducibility, allowed a precision of approximately 0.1 mg. per cent and an accuracy of approximately 0.25 mg. per cent in serum measurements of sulfonylurea.

Additional data relevant to the application of the serum sulfonylurea method are provided in TABLE 2. Chlorpropamide and tolbutamide, as well as tolbutamide and the tolbutamide metabolite, are interrelated in terms of their relative absorbance intensity in the method. A low serum "blank" value was found.

TABLE 2
DATA RELEVANT TO THE APPLICATION OF THE SERUM SULFONYLUREA ANALYTICAL METHOD

Comparative quantitation data:

	Relative absorbancy (on a weight basis)	
Chlorpropamide	100.0	—
Tolbutamide	83.8	100.0
Tolbutamide metabolite	—	38.5

Serum "blank" values (55 different serum samples from 5 normal, 2 diabetic, and 2 other subjects; run in 22 sets):
0.22 \pm 0.54 mg./100 ml. (as chlorpropamide)

Method not corrupted by:

- (1) Products from hemolysis
- (2) Existence of the tolbutamide metabolite

Mean \pm standard deviation given for serum blank values.

The method is not corrupted by the products from hemolysis. Aliquots of 2 different blood samples devoid of sulfonylurea were briskly beaten with wooden applicators while the blood was clotting. The resultant deeply pigmented sera, when compared in analysis with nonhemolyzed "duplicates," were found to have increased in apparent sulfonylurea only by about 0.4 mg. per cent. The degree of hemolysis produced deliberately in this evaluation was approximately 10 times greater than any experienced throughout the investigation being described. Mention is made of this observation concerning hemolysis, since plasma and serum tolbutamide methods in current use have a demonstrated¹ or likely²⁻⁴ sensitivity to corruption by the products from hemolysis.

As an important consideration, the sulfonylurea method used in our studies is not corrupted by the existence of the tolbutamide metabolite⁵ (1-butyl-3-*p*-carboxyphenylsulfonylurea). The metabolic half life of the tolbutamide metabolite was determined by techniques similar to those described below for tolbutamide. It was found to be approximately 9 times (8.6) shorter than the metabolic half life of tolbutamide. The metabolite thus disappears from blood approximately 9 times faster than tolbutamide and exhibits absorbancy approximately 2½ times less than tolbutamide in this method (TABLE 2). It is unlikely, therefore, that measured serum sulfonylurea levels, after tolbutamide administration, could be more than about 4 per cent in error because of the presence of the metabolite.

Other determinations. Fasting blood glucose; serum sodium, potassium and chloride; and urinary nitrogen, creatinine, sodium, potassium, chloride

17-hydroxycorticoids, and 17-ketosteroids were determined by methods previously used in this laboratory.⁶

Subjects. Two normal and 2 diabetic subjects were admitted to the Metabolism Research Laboratory and maintained on constant diets. The 2 normal subjects (R. R., male, age 23, 70 kg.; and J. D., male, age 20, 69 kg.) received a diet that provided 200 mEq. sodium, 113 mEq. potassium, 17.3 gm. nitrogen, 320 gm. carbohydrate, and 2900 calories per day. One diabetic patient (A. R., female, age 62, 87 kg.) received 178 gm. carbohydrate and 2150 calories per day; the other (R. B., female, age 63, 73 kg.) received 150 gm. carbohydrate and 1400 calories per day.

Eleven diabetic patients, chosen because they responded poorly to tolbutamide, were studied in the Endocrinology and Metabolism Out-Patient Clinic of the University Medical Center.

Results

The disappearance of chlorpropamide and tolbutamide from the blood. Measurements of chlorpropamide or tolbutamide disappearance from blood were made after intravenous administration of each compound. The series included observations on 2 normal and 2 diabetic subjects. After an overnight fast each subject received chlorpropamide or tolbutamide sodium* (30 mg./kg.) at 8:00 A.M., and food was withheld for 6 hours.

Data for one of the normal subjects are presented in FIGURE 1. It is seen that the chlorpropamide serum concentration declined from about 21 mg. per cent at 15 min. to about 18 mg. per cent at 6 hours, whereas the tolbutamide serum concentration fell more rapidly from about 20 mg. per cent at 15 min. to about 8 mg. per cent at 6 hours. The serum chlorpropamide concentration continued to decline slowly, and the serum tolbutamide concentration fell much more rapidly. The 2 drug levels extrapolated to zero concentration at 18 hours for tolbutamide and at 145 hours for chlorpropamide. Thus, under the circumstances of this experiment, tolbutamide disappeared from the blood approximately 8 times faster than chlorpropamide.

It is of interest to note (FIGURE 1) that there was no significant difference between the nature and degree of the acute hypoglycemia produced by the 2 drugs at this dosage level. Such was the case even though the tolbutamide serum concentration had fallen rapidly as compared with that of chlorpropamide. As would be anticipated, there was no correlation between the serum drug levels and the blood glucose levels in these acute (6-hour) experiments. It is likely that under these conditions hypoglycemia was maximally induced when the serum sulfonylurea levels (after the administration of either drug) were above 20 mg. per cent for 15 min., 18 mg. per cent for 30 min., or 17 mg. per cent for 45 min. Reversal of the progressive hypoglycemia at about 45 min. was undoubtedly the result of effective counteraction through physiological mechanisms that function to normalize the concentration of blood glucose.

FIGURE 2 shows data for one of the diabetic subjects. This subject had

* Chlorpropamide was supplied by D. Iezzoni, Chas. Pfizer & Co., Inc. Tolbutamide was supplied by C. J. O'Donovan, The Upjohn Co., Kalamazoo, Mich.

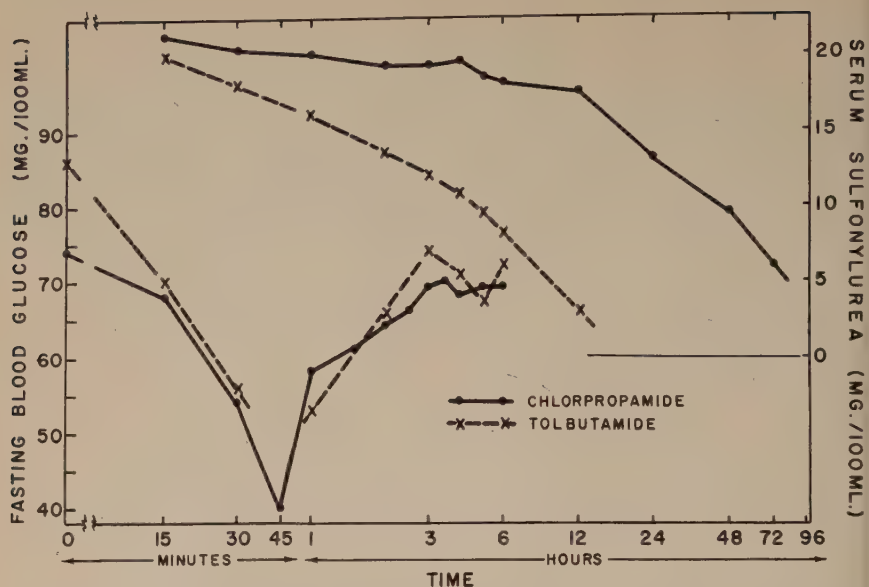


FIGURE 1. Serum sulfonylurea and fasting blood glucose levels after the intravenous administration of chlorpropamide and tolbutamide to a normal subject (J. D., male).

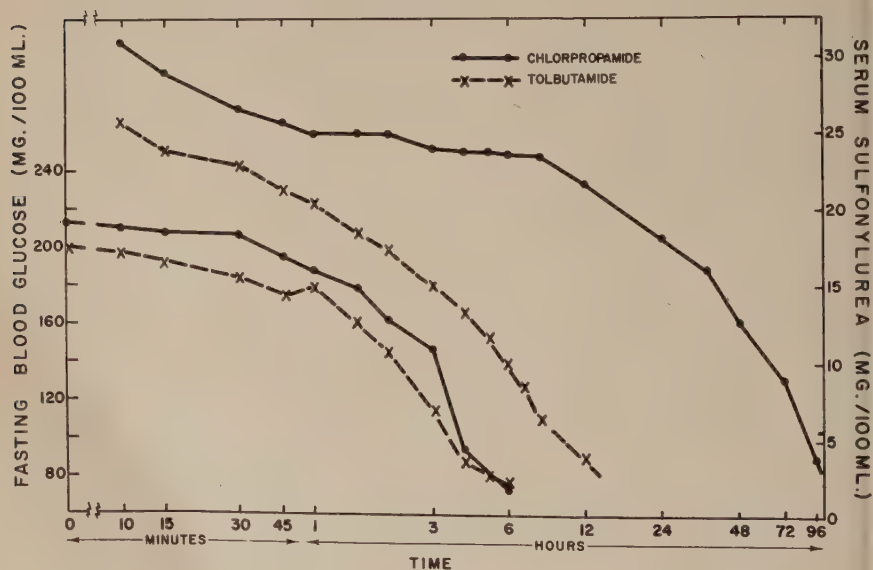


FIGURE 2. Serum sulfonylurea and fasting blood glucose levels after the intravenous administration of chlorpropamide and tolbutamide to a diabetic subject (A. R., female).

somewhat higher serum sulfonylurea levels during the study. The higher serum levels probably resulted from the fact that the dose administered was based upon gross body weight, and also from the fact that this subject was moderately obese. Another notable characteristic of the sulfonylurea disappearance curves, in this case, is the marked difference between the chlorpropamide and tolbutamide serum levels. Since the disappearance rates for the 2 drugs were very similar and quite constant during the interval from 10 through 60 min., the early difference in serum concentrations probably reflects a difference in distribution space for the 2 drugs.

In spite of the higher serum levels and the initial differential between the curves, the disappearance rates for chlorpropamide and tolbutamide in this diabetic subject were quite similar to those for the previous normal subject (FIGURE 1). It should be noted that this diabetic subject, as well as the other one in the series, was previously treated with both sulfonylurea compounds. The administration of chlorpropamide to both diabetic subjects was discontinued 9 days before these studies began.

In the diabetic subject (FIGURE 2) there were almost identical progressive reductions in blood sugar after intravenous administration of equal quantities of the 2 drugs. It is impressive that these acute blood glucose responses were remarkably similar in spite of the great difference that had developed in the serum sulfonylurea concentrations by 6 hours. These results are consistent with those obtained in the normal subject, and they suggest that the pattern for the blood glucose response was determined at an early time when the serum sulfonylurea concentration for each compound was sufficiently high to induce a maximal response.

In the other 2 subjects, the second normal and the second diabetic, there was also no significant difference in the acute blood glucose responses after intravenous administration of the indicated amounts of chlorpropamide and tolbutamide. The sulfonylurea disappearance rates for both of these subjects were very similar to those already illustrated (FIGURES 1 and 2).

A more detailed graphic evaluation of the disappearance rates of chlorpropamide and tolbutamide was made for each of the 4 subjects. Such an evaluation is illustrated in FIGURE 3 for one of the diabetic subjects. The disappearance rate is expressed as metabolic half life ($t_{1/2}$) which, conventionally, is defined as the time required for the serum concentration to decline to one half of a previous level.

It is seen that after chlorpropamide administration at zero time there occurs a very rapid decrease in the serum concentration during the first 10 min. (first 2 dots, at 5 and 10 min.). A rapid decline in serum concentration follows so that the metabolic half life of chlorpropamide during the time interval from 15 through 45 min. is calculated to be 2.8 hours. At about the first hour the serum concentration of chlorpropamide stabilizes and remains essentially unchanged up to the sixth hour. During this 5-hour period the half life of chlorpropamide is described, of course, as infinite. After this plateau phase, the half-life time fluctuates considerably until about 36 hours, when a terminal steady-state disappearance begins. During

this phase of terminal steady-state disappearance (36 to 144 hours) chlorpropamide is calculated to have a metabolic half life of 35 to 36 hours.

The disappearance of tolbutamide (FIGURE 3), after its intravenous administration, can be described also in rate phases. The three indicated phases for tolbutamide represent half life times of 3.3, 4.9, and 3.8 hours.

The phases which have been described in this subject for chlorpropamide and tolbutamide by half life calculations were also observed in the second diabetic and in both normal subjects. The simplest interpretation which can account for the phases observed after intravenous administration of each compound is: (1) an early (0 to 60 min.) unstable space distribution; (2) a redistribution (1 to 36 hours for chlorpropamide, 1 to 6 hours for tolbutamide); and (3) a stable space distribution (> 36 hours for chlorpropamide,

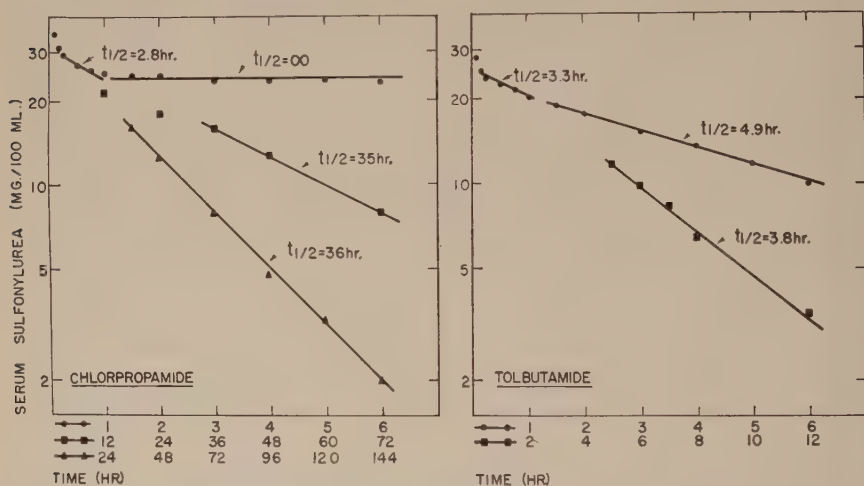


FIGURE 3. Metabolic half-life times for chlorpropamide and tolbutamide in a diabetic subject (A. R., female).

> 6 hours for tolbutamide). It would be extremely interesting to make space-distribution calculations for strategic timings in such an experimental series as this. Since our studies did not extend to drug balance (excretory studies), this was not possible for these subjects. It was interesting, however, to make initial or zero-time calculations of space distribution in a comparison of the 2 drugs. Extrapolation from the 5- and 10-min. points, in the 2 diabetic subjects gave chlorpropamide space volumes of 7.6 and 6.9 l. and tolbutamide space volumes of 9.0 and 9.3 l. The mean chlorpropamide and tolbutamide spaces in these 2 diabetics were 9 and 11 per cent of body weight, respectively.

The difference between mean values of 7.3 and 9.2 l. for chlorpropamide and tolbutamide spaces, respectively, would seem highly significant in these 2 subjects. From preliminary studies of deliberate blood sample hemolysis after chlorpropamide and tolbutamide administration it appears that the different space distributions of these 2 drugs may be explainable, to a major

extent, on the basis of different concentrations in red cell water. The calculated zero-time distribution volumes are probably smaller than the equilibrium distribution volumes and are discussed here only because they point up a difference between the 2 drugs. On the basis of published data by Wick *et al.*⁷ for tolbutamide in the eviscerated and nephrectomized rabbit, the equilibrium tolbutamide space might be anticipated as 13 to 19 per cent of body weight in man. Zero-time distribution calculations have not been made for the 2 normal subjects, since 5- and 10-min. points on the sulfonyl-urea disappearance curves were not obtained.

TABLE 3

METABOLIC HALF-LIFE MEASUREMENTS FOR CHLORPROPAMIDE AND TOLBUTAMIDE IN TWO NORMAL AND TWO DIABETIC SUBJECTS

Phase	Time interval (hours)	Metabolic half-life time (hours)					
		Normal subject			Diabetic subject		
		J. D.	R. R.	Mean \pm M. D.	A. R.	R. B.	Mean \pm M. D.
Chlorpropamide							
A	<1	3.0	2.9	3.0 ± 0.1	2.8	2.8	2.8 ± 0.0
B	1 to 6	∞	∞	∞	∞	∞	∞
C	36 to 72	36	35	} 35 ± 1	35	37	} 35 ± 1
	72 to 120	—	—		36	37	
Postoral	24 to 120	35	34		32	—	
Tolbutamide							
A	<1	1.8	1.8	1.8 ± 0.0	3.3	2.6	3.0 ± 0.4
B	1 to 6	5.1	6.1	5.6 ± 0.5	4.9	6.5	5.7 ± 0.8
C	6 to 12	4.3	4.8	4.6 ± 0.3	3.8	5.8	4.8 ± 1.0

Chlorpropamide/tolbutamide disappearance ratio: Normal subjects, $35/4.6 = 7.6$; diabetic subjects, $35/4.8 = 7.3$.

The disappearance data for all 4 subjects in the series are summarized in TABLE 3. A, B, and C represent the 3 phases previously described for intravenous chlorpropamide and tolbutamide in conjunction with FIGURE 3. There are probably no significant differences in the metabolic half-life times between the 2 normal and the 2 diabetic subjects for any of the 3 phases with each of the drugs, with the possible exception of the tolbutamide phase A.

Half-life times calculated after the cessation of oral (postoral) chlorpropamide administration are given in TABLE 3 for 3 of the 4 subjects as well.

The phase C and postoral metabolic half-life times, which represent terminal steady-state disappearance and a wide range of serum concentrations,

were used to arrive at a comparison of the rates of disappearance of these 2 drugs from the blood. This comparison is given as a ratio calculation below TABLE 3 and indicates that tolbutamide disappears from the blood from 7 to 8 times faster than chlorpropamide.

The relative potency of chlorpropamide and tolbutamide. It was clear that acute studies and classic dose-response studies would yield information of limited value in a potency comparison of chlorpropamide and tolbutamide especially if one attempted to apply such information in terms of long-term oral therapy. On the other hand, it appeared as though meaningful data

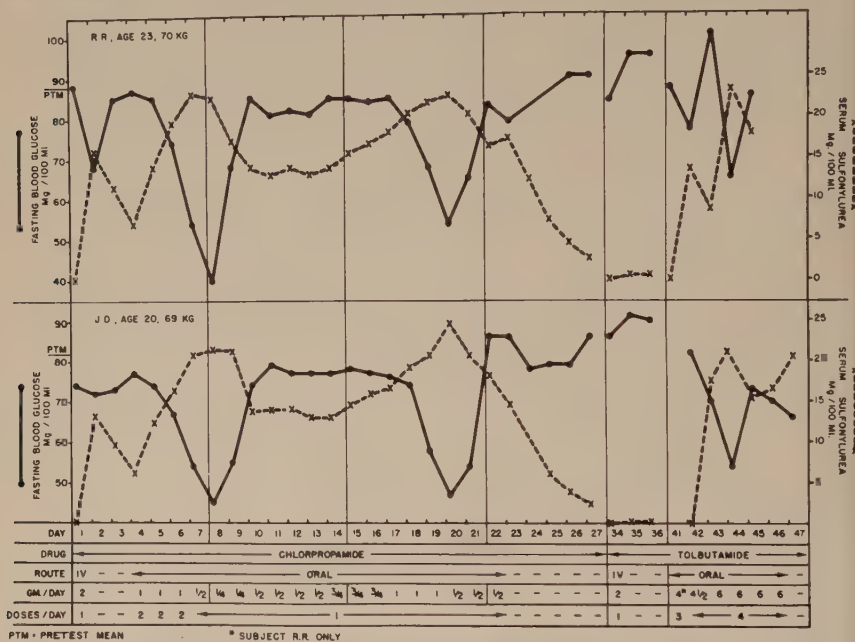


FIGURE 4. Fasting blood glucose and fasting serum sulfonylurea levels in 2 normal men during the administration of chlorpropamide and tolbutamide.

could be derived from long-term comparisons of the 2 drugs at various dosage levels and from a separation of the classic dose-response study into 2 distinct components: the relation of dose to drug blood level and the relation of drug blood level to blood glucose response. Such an approach would take into account the facts that: (1) the 2 drugs had markedly different half-life times, and (2) the 2 drugs probably had different spaces of distribution. With these considerations in mind, 2 normal men were studied for a period of about 4 weeks on an identical schedule of changing doses of oral chlorpropamide (FIGURE 4). During this period the men were carried through 2 cycles of symptomatic hypoglycemia. In a subsequent period they were observed during episodes of tolbutamide-induced hypoglycemia. Much larger oral dosages of tolbutamide (at least 6 times larger) were required to produce comparable hypoglycemia and comparable serum sul-

fonylurea levels. Daily measurements of the fasting blood glucose and the fasting serum sulfonylurea levels during oral administration of the 2 drugs revealed a consistent pattern.

In FIGURE 5 the fasting serum sulfonylurea levels are plotted against changes in the fasting blood glucose levels for the 2 normal men. Standard

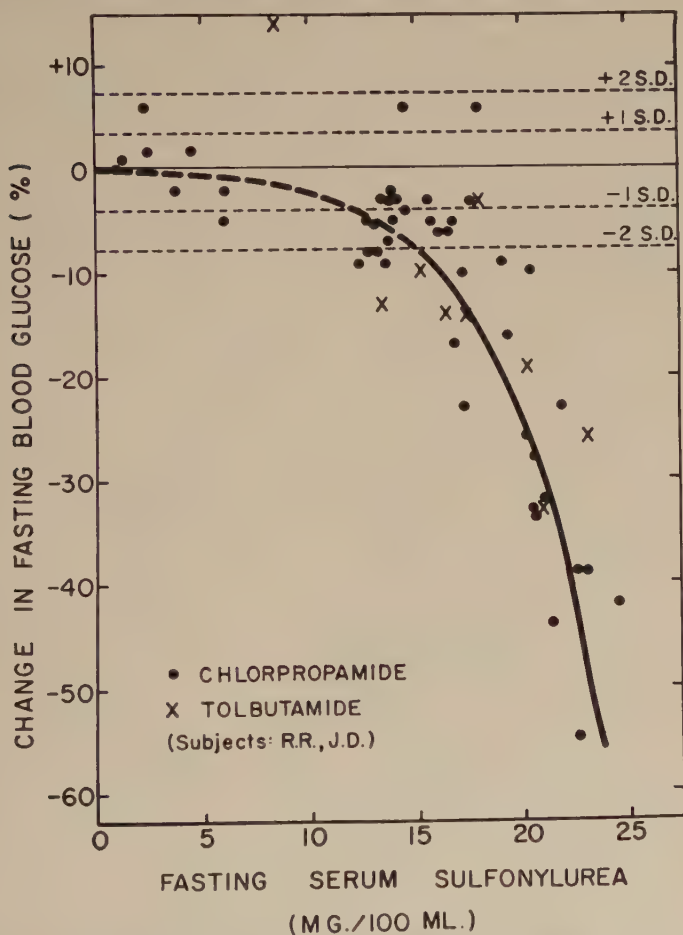


FIGURE 5. Correlation between change in fasting blood glucose and fasting serum sulfonylurea levels in 2 normal subjects.

deviations relate to both men for daily fasting blood glucose measurements during an 8-day pretest period. The curve was originally constructed in the absence of the tolbutamide points, with reference only to the chlorpropamide points. It is apparent, however, that when the tolbutamide points are included, as they are in this figure, they fit the previously constructed curve for chlorpropamide. These results indicate that, within the standardizations and extensiveness of these observations, chlorpropamide

and tolbutamide are equally active, on the basis of serum concentrations, as agents to produce change in the blood glucose concentration.

A serum sulfonylurea level of about 12 mg. per cent was required to produce a decrease of 1 standard deviation in the fasting blood glucose in these normal men. A decrease of twice the standard deviation in the fasting blood glucose resulted from a serum sulfonylurea level of about 15 mg. per

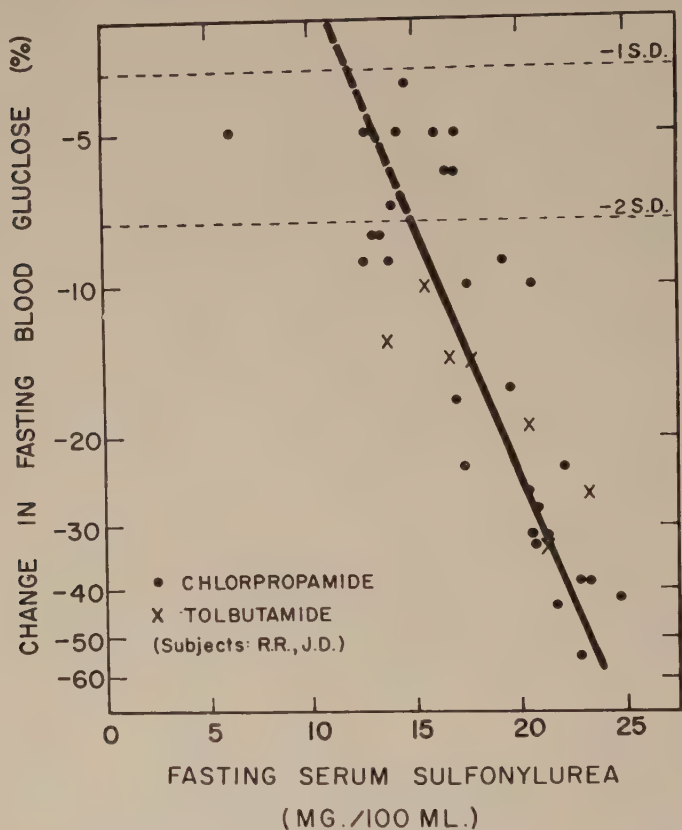


FIGURE 6. Correlation between change in fasting blood glucose and fasting serum sulfonylurea levels in 2 normal subjects (semilogarithmic presentation).

cent. All sulfonylurea levels above 18.5 mg. per cent produced decreases in the fasting blood glucose of at least twice the standard deviation. Within the range of significant measurements, increments in serum sulfonylurea concentration of approximately 3 mg. per cent double the effect on the fasting blood glucose.

The data from FIGURE 5 are reproduced in FIGURE 6 with semilogarithmic coordinates. Under this circumstance the relationship between the fasting serum sulfonylurea level and the change in the fasting blood glucose is represented by a straight line.

Since chlorpropamide and tolbutamide, on the basis of their serum concentrations, are approximately equally active, and since tolbutamide disappears from the blood approximately 8 times faster than chlorpropamide, it follows that chlorpropamide has the potentiality of being approximately 8 times more effective than tolbutamide, on a weight basis, when the 2 drugs are used as oral therapeutic agents.

It was of interest to obtain information on how the serum chlorpropamide concentration varies during a 24-hour period when 500 to 750 mg. is administered each morning with breakfast (TABLE 4). Although the 2 subjects had

TABLE 4

VARIATION OF SERUM CHLORPROPAMIDE LEVELS DURING THE DAY AFTER SINGLE ORAL MORNING DOSES OF THE DRUG IN 2 NORMAL MEN

Subject	Day*	Chlorpropamide dose (gm./day)†	Time	Serum chlorpropamide (mg./100 ml.)	Per cent of initial fasting level
R. R. (70 kg.).....	15	0.75	8:00 A.M.	15.7	100
			10:00 A.M.	23.5	150
			12:00 N.	24.6	157
			2:00 P.M.	21.4	136
	16	0.50	8:00 A.M.	16.8	107
	20		8:00 A.M.	22.7	100
			3:00 P.M.	27.2	120
	21	0.50	8:00 A.M.	20.3	89
	21		8:00 A.M.	20.3	100
			5:00 P.M.	24.6	121
	22			8:00 A.M.	16.5
J. D. (69 kg.).....	15	0.75	8:00 A.M.	14.6	100
			10:00 A.M.	16.0	110
			12:00 N.	18.0	123
			2:00 P.M.	17.0	116
	16	0.50	8:00 A.M.	15.8	108
	20		8:00 A.M.	24.6	100
			4:00 P.M.	26.8	109
	21	0.50	8:00 A.M.	20.6	84
	21		8:00 A.M.	20.6	100
			5:00 P.M.	21.2	103
	22			8:00 A.M.	18.2

* Days correspond with those in FIGURE 4.

† Total dose taken shortly after 8:00 A.M. with breakfast.

very similar fasting serum chlorpropamide concentrations on the same drug dosages, they showed somewhat different but quite consistent variations in serum levels during the intervals studied. The subjects developed maximum serum chlorpropamide concentrations of 123 and 157 per cent of the fasting levels about 4 hours after an oral dose at 8:00 A.M. The serum levels at 5:00 P.M. were 103 and 121 per cent of the morning fasting levels even when there was a moderate decline in the fasting serum level the following morning.

Thus, a single dose of chlorpropamide at 8:00 A.M. would seem to provide elevated serum sulfonylurea levels (that is, relative to the fasting level) during the absorptive portion of the day (about 8:00 A.M. to 8:00 P.M.) and to allow the serum level to decline to a near-fasting level early in the usually non-absorptive portion (about 8:00 P.M. to 8:00 A.M.) of the day. This feature, which is provided by the long metabolic half-life time of chlorpropamide, may prove to be advantageous therapeutically.

Clinical studies. Eleven diabetic patients who responded poorly to tolbutamide were treated with chlorpropamide. All responded better to chlorpropamide at considerably lower daily dosages in most cases.

The comparative response of the 11 diabetic patients to the 2 compounds is illustrated in TABLE 5. Each patient received each substance, at the indicated dosages, for at least 3 days and usually for 1 week prior to the determinations. Complete comparative data were obtained for the first 3 patients

TABLE 5
COMPARATIVE RESPONSE OF PATIENTS WITH DIABETES MELLITUS TO ORAL
TOLBUTAMIDE AND CHLORPROPAMIDE THERAPY

Patient	Tolbutamide			Chlorpropamide		
	Dosage (gm./day)	Fasting blood glucose (mg./100 ml.)	Fasting serum tolbutamide (mg./100 ml.)	Dosage (gm./day)	Fasting blood glucose (mg./100 ml.)	Fasting serum chlorpropamide (mg./100 ml.)
A. R. (95 kg.)..	3.0	191	5.3	0.75	138	14.2
E. E. (74 kg.)..	1.5	183	6.1	0.5	86	19.5
H. H. (60 kg.)..	3.0	176	4.6	1.0	88	29.3
R. B. (73 kg.)..	2.0	150	—	1.0	136	22.0
A. C. (58 kg.)..	2.0	210	—	2.0	146	—
F. F. (55 kg.)..	2.0	168	—	1.0	131	16.1
K. H. (72 kg.)..	1.0	155	—	0.5	83	22.2
S. J. (107 kg.)..	3.0	227	—	1.5	155	36.3
M. R. (88 kg.)..	1.5	153	—	0.5	109	10.2
P. S. (41 kg.)..	1.5	210	—	1.0	139	35.5
N. M. (75 kg.)..	1.0	131	—	0.25	99	7.5

listed. The decreases in the fasting blood glucose levels experienced by these 3 patients on the indicated tolbutamide dosages were slight. The relatively low fasting serum tolbutamide levels are to be noted. In the same 3 patients considerably lower dosages of chlorpropamide produced higher serum levels of the drug and, concurrently, more intense decreases of the fasting blood glucose levels. The same pattern of response described for the first 3 patients is characteristic of the other 8, as well.

The data presented for these 11 diabetic patients are consistent with the hypothesis that neither drug is intrinsically more effective than the other, and that the greater clinical efficacy of chlorpropamide, as compared with tolbutamide, is due to its longer metabolic half life. The longer metabolic half life (that is, the slower disappearance from the blood) enables the production of higher and more sustained serum sulfonylurea levels on a given dosage schedule.

TABLE 6
METABOLIC BALANCE AND RENAL EXCRETION STUDIES IN TWO NORMAL MEN DURING CHLORPROPAMIDE ADMINISTRATION*

Subject	Period	Day†	F.B.S. (mg./ 100 ml.)	Urine						
				Gm./24 hours		mEq./24 hours			Mg./24 hours	
				Creatinine	Nitrogen	Na	K	Cl	17-HCS	17-KS
R. R. (Age 23, 70 kg.) . .	Control	-8-0	88 (81 to 91)	2.22 (2.06 to 2.30)	14.7 (12.9 to 17.3)	179 (138 to 218)	111 (87 to 134)	193 (163 to 232)	10.0 (8.8 to 11.2)	17 (15 to 20)
	Chlorprop- amide	1-28	— (40 to 90)	2.12 (1.98 to 2.23)	13.0 (10.3 to 14.7)	186 (104 to 246)	108 (58 to 145)	201 (119 to 280)	10.2 (8.4 to 16.2)	16 (12 to 22)
	Control	29-33	91 (87 to 95)	2.07 (1.81 to 2.16)	13.3 (12.5 to 13.8)	170 (146 to 202)	104 (84 to 115)	212 (202 to 226)	9.3 (7.4 to 10.0)	19 (15 to 20)
J. D. (Age 20, 69 kg.) . .	Control	-8-0	82 (79 to 85)	2.34 (2.25 to 2.42)	15.2 (13.4 to 16.4)	180 (165 to 193)	114 (103 to 130)	201 (186 to 218)	8.0 (7.1 to 8.6)	20 (18 to 21)
	Chlorprop- amide	1-28	— (45 to 86)	2.13 (1.75 to 2.30)	13.2 (10.0 to 14.9)	183 (117 to 242)	108 (60 to 156)	202 (144 to 266)	7.9 (5.2 to 13.5)	18 (15 to 22)
	Control	29-33	87 (85 to 89)	2.16 (2.09 to 2.25)	12.6 (12.0 to 13.0)	187 (166 to 223)	96 (81 to 122)	213 (187 to 248)	6.2 (5.8 to 6.5)	18 (15 to 20)

* Days correspond with those in figure 4.

† Values represent mean and range of daily determinations.

An example of the difference in response to the 2 drugs is illustrated in FIGURE 7.

Metabolic balance studies. Two normal men were studied for a period of about 4 weeks on an identical schedule of changing doses of chlorpropamide. The balance and renal excretion studies (TABLE 6) during chlorpropamide administration yielded results similar to those previously published from this laboratory for tolbutamide and carbutamide.⁶

There were no significant changes from control averages, for the mean urinary excretion of nitrogen, creatinine, electrolytes, 17-hydroxycorticoids, and 17-ketosteroids, while on chlorpropamide. However, on several individual

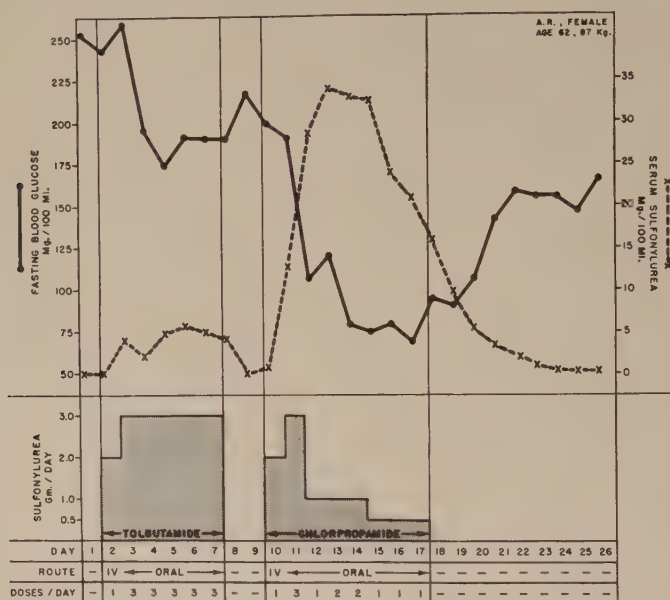


FIGURE 7. Fasting blood glucose and fasting serum sulfonylurea levels in a diabetic subject during the administration of tolbutamide and chlorpropamide.

days when severe symptomatic hypoglycemia had been produced, urinary excretion of nitrogen and electrolytes was significantly decreased, and the excretion of 17-hydroxycorticoids was increased. Serum levels of sodium, potassium, and chloride likewise were not affected.

Summary and Conclusions

(1) A single analytical method has been found to perform well for the determination of both chlorpropamide and tolbutamide in serum.

(2) Evidence has been presented from long-term studies to indicate that, on the basis of serum concentrations, chlorpropamide and tolbutamide are approximately equally active in normal men.

(3) Tolbutamide disappears from the blood of normal men and diabetic subjects approximately 8 times faster than does chlorpropamide.

(4) Chlorpropamide, when compared with tolbutamide on a weight basis, exhibits a markedly greater clinical effectiveness.

(5) The greater clinical efficacy observed with chlorpropamide seems adequately explainable on the basis of the capacity of the drug to produce and maintain higher serum levels on a given dosage schedule.

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A COMPARISON OF THE ACUTE HYPOGLYCEMIC POTENCIES OF TOLBUTAMIDE AND CHLORPROPAMIDE*

James W. Craig, Max Miller, Frank D. Mills, Naomi Nickerson

Department of Medicine, the School of Medicine, Western Reserve University, Cleveland, Ohio

Comparisons of the clinical effectiveness of tolbutamide and chlorpropamide during their chronic administration to diabetic patients have indicated that smaller doses of chlorpropamide are required to produce a similar lowering of the blood glucose concentration. At least part of this greater clinical potency of chlorpropamide as compared to tolbutamide is explained by its slower rate of disappearance from the body. In the current studies the inherent hypoglycemic potencies of tolbutamide and chlorpropamide have been compared in terms of the acute decreases in blood glucose produced by the intravenous administration of equal doses of the two drugs to the same diabetic patients. Use of the intravenous rather than the oral route of administration avoided possible differences in the rates of gastrointestinal absorption of the two compounds, and the acute nature of the study permitted a comparison of the effects of the drugs before the difference in their half lives had become manifest in significantly different plasma drug levels.

Each patient was tested twice, once with tolbutamide† and on another occasion with an equal dose of chlorpropamide‡. The patients were free of the influence of any hypoglycemic agent; that is, they had received no long-acting insulin for at least 48 hours, no regular insulin for 15 hours, no tolbutamide for 15 hours, and no chlorpropamide for at least 96 hours before any of the tests. Preceding the tests the patients rested in bed after an overnight fast. A 5 per cent aqueous solution of the sodium salt of the drug was injected into a peripheral vein within 1 min. Blood samples were obtained through an indwelling needle in a peripheral vein before and at regular intervals after drug administration for a total of 3 hours (TABLES 1 and 2). Blood glucose was determined by the method of Somogyi,¹ and plasma chlorpropamide and tolbutamide by the method of Toolan and Wagner.² The statistical significance of differences between responses to the two drugs was assessed by analysis of the mean differences of paired observations.

As shown in TABLE 1, equal doses of tolbutamide and chlorpropamide produced similar decreases in the blood glucose concentration during the 3 hours after drug administration. The differences between these declines were not statistically significant. The plasma concentrations of the drugs are shown in TABLE 2. Although at 2 and 3 hours the average tolbutamide levels were slightly less than the chlorpropamide levels, again the differences were not statistically significant.

* The study reported here was supported in part by the Diabetes Research Program of Western Reserve University, Cleveland, Ohio, through Research Grant A-1317 from the National Institute of Arthritis and Metabolic Diseases, Public Health Service, Bethesda, Md.; by the Fund for the Study of Diabetes Mellitus and Related Metabolic Diseases, Western Reserve University; and by the John A. Hartford Foundation, Inc., New York, N. Y.

† Supplied by The Upjohn Company, Kalamazoo, Mich.

‡ Supplied by Chas. Pfizer & Co., Inc., Brooklyn, N. Y.

TABLE 1
MEAN DECREASES IN BLOOD GLUCOSE CONCENTRATION PRODUCED BY THE INTRAVENOUS ADMINISTRATION OF EQUAL QUANTITIES OF SODIUM TOLBUTAMIDE (T) OR SODIUM CHLORPROPAMIDE (C) TO THE SAME DIABETIC PATIENTS

Dose of drug (gm.)	No. of subjects	Drug administered	Fasting blood sugar (mg./100 ml.)	Blood glucose decrease (mg./100 ml.)					
				Minutes after administration					
				30	60	90	120	150	180
1.0	8	T C T-C*	187	11.5	20.2	32.8	41.1	—	55.4
			173	9.7	23.2	30.5	41.3	—	55.7
			—	1.8 ± 8.4	-3.0 ± 4.7	2.3 ± 5.4	-0.2 ± 6.2	—	-0.3 ± 5.7
0.5	4	T C T-C*	213	—	15.0	17.8	27.7	—	48.0†
			206	13.5	22.0	32.0	38.2	—	59.5†
			—	—	-7.0 ± 9.5	-14.2 ± 7.0	-10.5 ± 7.8	—	-11.5†
0.25	3	T C T-C*	134	11.3	19.3	29.7	30.6	—	26.5
			133	—	15.3	19.3	23.0	—	27.0
			—	—	4.0	10.4	7.6	—	-0.5
0.125	2	T C T-C*	202	6.5	15.0	23.5	31.0	33.5	39.0
			202	4.0	—	15.0	20.5	27.0	38.0
			—	2.5	—	8.5	10.5	6.5	1.0

* Mean difference ± one standard error between blood glucose decreases after tolbutamide and decreases after chlorpropamide.

† Data from only 2 of the 4 subjects.

TABLE 2
MEAN PLASMA CONCENTRATIONS OF TOLBUTAMIDE (T) AND CHLORPROPAMIDE (C)
AFTER INTRAVENOUS ADMINISTRATION OF EQUAL QUANTITIES OF THE SODIUM
SALTS OF THE DRUGS TO THE SAME DIABETIC PATIENTS

Dose of drug (mg.)	No. of subjects	Drug administered	Concentration of drug in plasma (mg./100 ml.)		
			Minutes after administration		
			60	120	180
1.0	8	T	9.9	7.8	6.4
		C	8.8	8.6	8.1
		T-C*	1.1 ± 1.2	-0.8 ± 1.8	-1.7 ± 1.1
0.5	4	T	5.8	4.5	3.8†
		C	7.2	5.0	4.8†
		T-C*	-1.4 ± 1.3	-0.5 ± 0.2	—
0.25	3	T	3.0	2.0	1.5
		C	2.5	2.4	1.9
		T-C*	0.5	-0.4	-0.4
0.125	2	T	2.8‡	0.7	0.5
		C	1.2§	1.1	—
		T-C*	1.6	-0.4	—

* Mean difference \pm one standard error between plasma concentrations of tolbutamide and chlorpropamide.

† Only 2 values included.

‡ One of samples obtained at 90 min.

§ Both samples obtained at 90 min.

Thus, at similar blood levels, the two drugs produced a similar fall in blood glucose during the first three hours after their intravenous administration to diabetic patients. These data cannot be applied directly to the chronic administration of the drugs; however, Knauff *et al.*³ found during long-term administration of tolbutamide and chlorpropamide that the degree of fasting hypoglycemia was a function of the fasting sulfonylurea level and that the same relationship applied to both drugs. These findings suggest that the lower dosage requirement for chlorpropamide as compared to tolbutamide is due to the longer half life of chlorpropamide rather than to any difference in the inherent hypoglycemic potency of the two drugs.

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Part III. Clinicometabolic Studies

STUDIES WITH CHLORPROPAMIDE IN DIABETIC PATIENTS*

William B. Hadley

Joslin Clinic and New England Deaconess Hospital, Boston, Mass.

Avedis Khachadurian†

Baker Clinic Research Laboratory, New England Deaconess Hospital

Alexander Marble

Joslin Clinic, New England Deaconess Hospital, and Harvard Medical School, Boston, Mass.

The clinical trials of chlorpropamide reported here were begun in April 1958; our experience to date therefore extends over only 5 months. The studies were initiated with the following four objectives in mind: first, to determine the effectiveness of the drug as a hypoglycemic agent in the management of diabetes, second, to observe serum levels of the compound in patients under control, third, to determine whether or not long-term effectiveness could be predicted by the blood sugar response in a single-dose test and, finally, to study the possible toxicity of the drug by performing serial liver and kidney function tests and hematological studies.

Clinical Material and Methods

Seventy-eight patients with proved diabetes, selected primarily on the likelihood of responsiveness to sulfonylurea compounds, have been given chlorpropamide to date. The group consisted of 48 females and 30 males. The ages of this group varied between 35 and 83 years, and all but 10 were above 50 years of age. The age at onset of diabetes varied from 23 to 77 years; in 10 patients this had been below the age of 40 years. The duration of diabetes varied from a few weeks to 21 years; in 60 patients this was more than 1 year.

In all cases but one the patients were hospitalized and maintained on weighed diets. The total amount of sugar excreted in the urine was determined for each 24-hour period. The blood sugar was determined frequently, usually 3 times daily, before breakfast, at 11 A.M., and at 3 P.M. The majority of the patients were ambulatory. The diets provided from 130 to 200 gm. of carbohydrate daily, with most in the range of 150 to 170 gm. The caloric content varied from 1000 to 2000 calories daily; often this was low so as to favor weight reduction. In all but 21 patients the insulin requirement had been established either by long-term usage or during a brief period of regulation prior to trial of the oral hypoglycemic agent. In the 57 patients whose insulin requirement was known, this was 40 units or below in 47 patients and 20 units or below in 22 patients.

Blood sugar determinations were carried out by the Somogyi-Nelson procedure. Most blood sugar levels before breakfast were determined on venous blood; most other samples were capillary. For the determination

* The work reported in this paper was supported in part by grants from Chas. Pfizer & Co., Inc., Brooklyn, N. Y., and The Upjohn Company, Kalamazoo, Mich.

† Research Fellow.

of the level of chlorpropamide we used the method of Spingler,¹ as modified by R. H. Carmichael. In this procedure serum is extracted with isoamyl acetate and treated with 1-fluoro-2-4-dinitrobenzene and the resulting color measured in the Beckman spectrophotometer at 345 m μ . Prior to the beginning of therapy most patients had base line liver function studies, including thymol turbidity and flocculation tests, alkaline phosphatase determinations, and Bromsulphalein retention tests. At each follow-up examination a urine analysis, including examination of the sediment, was performed. Liver function studies are being done after each 3 months of treatment with the drug.

Sulfonylurea response test. Prior to long-term trial with chlorpropamide a single-dose response test was done. In general, the procedure used was the same as that previously described in studies with carbutamide and tolbutamide.² The test was carried out as follows: (1) long-acting insulin was omitted for 24 hours prior to the test day, with crystalline insulin being substituted where necessary; and (2) at 8 A.M. on the day of the test 1.0 gm. of chlorpropamide was given orally after an overnight fast, and glucose determinations were done on 3 blood samples: (a) fasting, before chlorpropamide; (b) fasting, 12 noon; and (c) at 3 P.M. (about 2½ hours after a regular noon meal).

The maintenance dose in almost all patients was either 0.25 or 0.50 gm./day. A few patients received 1 gm./day for short periods during the early period of the study.

Results

Response tests. The response test was carried out in 71 patients. In 37 a fall in blood sugar of 20 per cent or more occurred at both 4 and 7 hours. Fifty-three patients showed a similar fall only at 4 hours, 5 patients only at 7 hours, and 10 patients failed to show a response at either time interval.

Maintenance. Seventy-two patients were maintained on the drug for periods varying from a few days to 140 days. Thirty-eight patients received the drug for periods longer than one month and 48 for more than 10 days.

The results of maintenance were graded as good, fair, and poor according to previously established criteria.² Forty-nine patients (68 per cent) achieved good control; in 11 patients (15 per cent) the response was graded as fair; and in 10 patients (14 per cent) poor results were noted. Two patients with good initial control later failed to respond (secondary failures).

Chlorpropamide therapy was discontinued in 36 patients for the following reasons: poor control on maintenance, 12; untoward or toxic effects, 2; diet alone adequate, 11; nonmedical reasons, 11.

The average random blood glucose in 36 patients still receiving the drug as of the last follow-up was 93 mg./100 ml., with a range of 56 to 137 mg./100 ml.

Serum chlorpropamide levels determined on 31 blood samples from 24 patients ranged from 3 to 32 mg./100 ml., with an average of 10 mg./100 ml. The average values for sera taken at 4, 7, and 24 hours after the initial 1-gm. test dose were 9.4, 9.2, and 8.7 mg./100 ml., respectively.

Minor toxic effects consisted of minimal gastrointestinal symptoms in

2 patients and a skin rash in another. One patient developed jaundice after 3 weeks of therapy with 0.50 gm./day. He was hospitalized, and initial laboratory studies showed the following results: bilirubin, total, 3.9 and direct, 3.0 mg./100 ml.; Bromsulphalein retention, 49 per cent at 45 min.; alkaline phosphatase, 19.2 Bodansky units; thymol turbidity, 0.3 units; and cephalin and thymol flocculations negative. The differential white blood cell count showed an eosinophilia of 10 per cent, falling to 1 per cent after 12 days. Liver biopsy showed canalicular bile stasis, but no evidence of hepatocellular damage. The patient improved with conservative therapy, and the last follow-up 2 months later showed the following laboratory results: bromsulphalein retention, 9 per cent in 45 min.; alkaline phosphatase, 4.7 Bodansky units; thymol turbidity, 0.8 units; and thymol flocculation negative.

Discussion

Of the 50 patients who showed a fall of more than 20 per cent in blood glucose in the 4-hour sample of the response test, 41 achieved good control. Of the 31 patients showing a fall of 20 per cent or more in both 4- and 7-hour samples, 26 achieved good control. These results, in conjunction with the finding of an adequate serum level of chlorpropamide at 4 hours, suggest that the response test carried over 7 hours has no advantage over the 4-hour test. Eighty per cent of patients having a satisfactory response with either test are likely to have their diabetes controlled. Of the 13 patients who had less than a 20 per cent fall at 4 hours, 7 achieved good control, 2 fair control, and 4 poor control. It thus seems that a poor response test does not preclude subsequent control of diabetes with chlorpropamide.

The serum chlorpropamide determinations suggest that adequate levels are attained within 4 hours and maintained over 24 hours after an initial dose of 1 gm., and throughout longer periods with doses of 0.25 to 0.50 gm./day.

In evaluating the effectiveness of chlorpropamide in controlling diabetes, the role of diet must be considered. As most of our patients were obese, their diets were restricted in calories as well as carbohydrate content. The average weight loss of our patients during this study period was 2.9 kg., and in 11 patients chlorpropamide could be discontinued and control maintained with diet alone.

The only serious toxic manifestation in our group of patients was the occurrence of jaundice in a 54-year-old man. The clinical picture, the laboratory data, and the histological appearance of a punch biopsy of the liver were all similar to findings in jaundice produced by chlorpromazine and methyltestosterone. It is noteworthy, however, that our patient had a past and family history of jaundice, the nature of which was not determined.

Summary and Conclusions

(1) Seventy-eight patients, selected mainly on the likelihood of responsiveness to sulfonylurea compounds, were given chlorpropamide. All but 10 were above 50 years of age, and the onset of diabetes occurred before the age of 40 in 10 patients.

(2) In 71 patients the response of the blood sugar to 1 gm. of chlorpropamide was determined at 4 and 7 hours. Eighty per cent of the patients who had a fall of 20 per cent or more at 4 hours subsequently achieved good control of hypoglycemia and glycosuria with maintenance doses. Of the 72 patients who received the drug for a period of up to 140 days, 68 per cent achieved good control, 15 per cent fair control, and 14 per cent poor control.

(3) Serum chlorpropamide levels ranged between 3 and 32 mg. per 100 ml., with an average of 10 mg. per 100 ml.

(4) One patient with a past and family history of jaundice developed an obstructive type of jaundice 3 weeks after onset of therapy. There was complete recovery. Two patients had minor gastrointestinal symptoms, and 1 patient developed a skin rash.

(5) These preliminary studies indicate that, in selected patients, chlorpropamide is an effective hypoglycemic agent. Wider experience with the drug is needed to evaluate its long-term usefulness and incidence of untoward effects.

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USE OF CHLORPROPAMIDE IN DIABETES MELLITUS IN USUAL DIABETIC PATIENTS AND IN PRIMARY AND SECONDARY TOLBUTAMIDE FAILURES*

Samuel J. N. Sugar, Lawrence J. Thomas

George Washington University School of Medicine, Washington, D. C.

Serkis Tatlier

Prince George's General Hospital, Cheverly, Md.

In a preliminary report¹ on management of diabetes with chlorpropamide (Diabinese†) successful control was noted in 58 per cent of 45 patients studied. Further observations were made in 70 cases for periods up to 8 months. We were particularly interested in observing the effectiveness of chlorpropamide in a group of patients who were primarily unresponsive to tolbutamide (within the first month of treatment) and in a larger series of cases classified as "secondary tolbutamide failures"; that is, patients in whom diabetes had slipped out of control after a variable period of satisfactory management with tolbutamide.

Method of Study

Diabetic patients who were taking insulin, an occasional patient not controlled with diet alone, and those not responding to tolbutamide were used in this investigation. The first few patients were hospitalized, but later the drug was given to clinic and ambulatory private patients. Diet was prescribed according to optimal caloric requirements.

In the hospital, fractional urinalyses for sugar and acetone were made by the ward nurses before meals and at bedtime. Each test showing more than a trace of sugar (Clinitest‡ modification of Benedict's test) was covered by an injection of regular insulin ranging in dosage from 5 to 20 U. depending upon the percentage of sugar present. In this way we were able to prevent ketosis and at the same time observe the degree of effectiveness of chlorpropamide. Daily fasting blood sugar, an occasional blood sugar after a meal, and 24-hour quantitative urine glucose determinations were made as added parameters of control.²

Management with chlorpropamide was considered satisfactory if the average daily fasting blood sugar was substantially reduced and was in the region of 200 mg./100 ml. or less. Urine tests were required to be negative or show less than one fourth per cent sugar. When observation for at least 3 days indicated that these requirements had been attained, patients were given a supply of medication and discharged for follow-up in the outpatient clinic. Particular stress was laid on the need for frequent urine tests at

* The research reported in this paper was conducted in the Medical Department of Prince George's General Hospital, Cheverly, Md., and the Diabetic Clinic, District of Columbia General Hospital, George Washington University Division, Washington, D. C.

† The Diabinese used in this study was supplied by D. Iezzoni of Chas. Pfizer & Co., Inc., Brooklyn, N. Y.

‡ The Clinitest apparatus and tablets are manufactured by The Ames Co., Inc., Elkhart, Ind.

home, and a written record of the results was requested. On returning to the clinic, the patient was examined, home urine tests were reviewed, and the fasting blood sugar for that day determined. If most of the urine tests were reported negative for sugar and if the blood sugar was below 120 mg./100 ml., the dose of chlorpropamide was reduced by half. If the blood sugar was between 120 and 175, the same dosage was maintained. If it was above 175, the dose was increased by 0.25 gm. increments until the blood and urine glucose levels dropped to the above-mentioned control levels.

In a similar manner the patients not hospitalized were given the drug and requested to test the urine at home at least twice daily. Those taking insulin were given chlorpropamide concomitantly. Usually the dose of insulin was decreased by decrements of 10 U. as control was maintained by a constant dosage of chlorpropamide until eventually no insulin was needed. If the blood and urine tests showed increasing amounts of glucose, chlorpropamide was stopped and insulin dosage adjusted until control was again apparent. Patients were seen at weekly intervals until management was considered satisfactory; thereafter the frequency of visits was decreased as improvement progressed.

Those patients taking tolbutamide but showing persistent glycosuria and fasting blood sugar always above 200 mg./100 ml. were considered not adequately managed. They were placed on chlorpropamide and followed in the manner already described.

Dosage

Chlorpropamide was made available in uncoated tablets containing 0.5 gm. The first few patients were given an initial "priming" dose of 2.5 gm. As our studies continued, it was found that a starting dose of 1.0 gm./day in 2 doses, one given before breakfast and one before dinner, proved most effective. If control was not satisfactory after 1 week, 1.5 gm. was given. No patients benefited by increasing the dose to 2.0 gm. per day. The dose was decreased, usually by decrements of 0.25 gm., when good control, as manifested by lowered fasting blood sugar and negative urine tests, was noted.

Results

An analysis of 70 cases treated with chlorpropamide is presented in TABLE 1. Forty-two patients (60 per cent) studied for more than 2 months showed ade-

TABLE 1
ANALYSIS OF 70 CASES TREATED WITH CHLORPROPAMIDE

	No. treated	No. successful	Percentage
Usual diabetic patients.	29	20	69
Primary (early) tolbutamide failures.	10	2	20
Secondary (late) tolbutamide failures.	31	20	64
Totals.	70	42	60

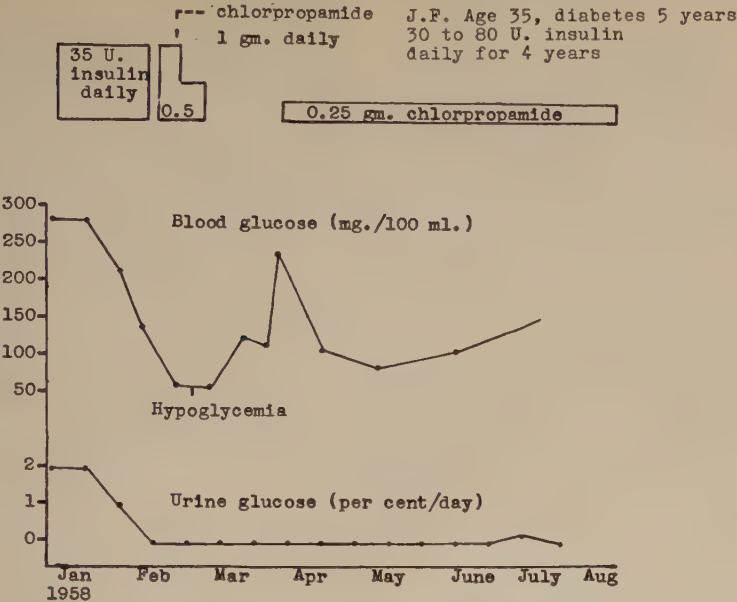


FIGURE 1. Good control with chlorpropamide in the usual diabetic patient.

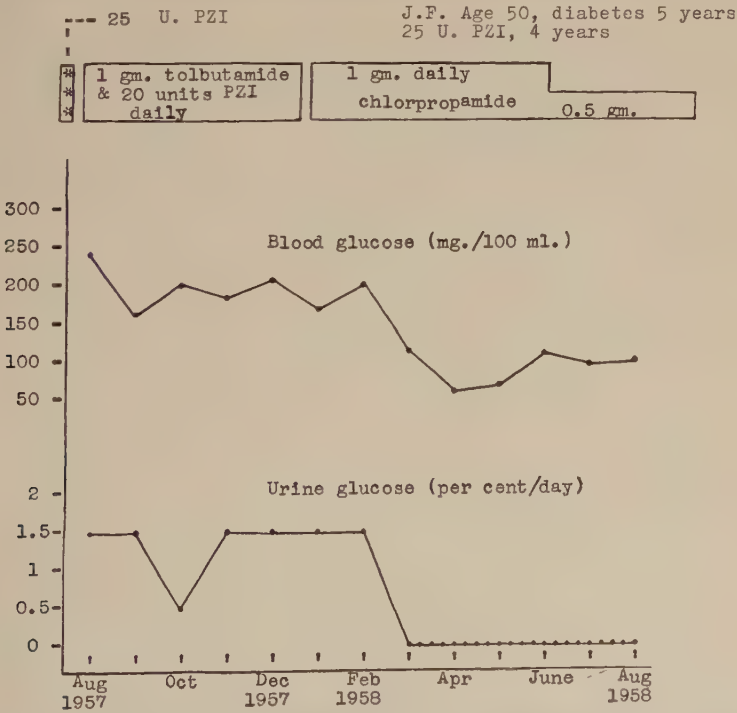


FIGURE 2. Good control with chlorpropamide after tolbutamide primary failure.

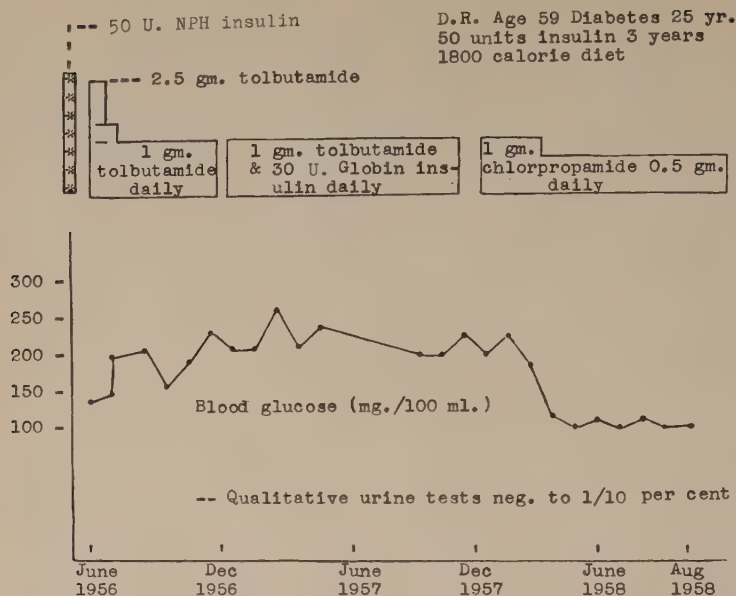


FIGURE 3. Good control with chlorpropamide after tolbutamide secondary failure.

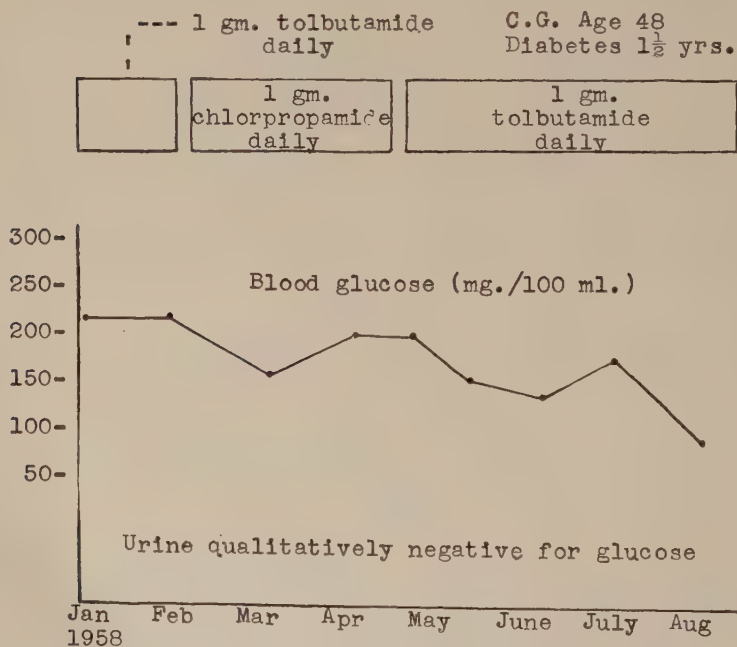


FIGURE 4. Good response to a second trial of tolbutamide after discontinuing chlorpropamide because of disagreeable taste.

quate control, as measured by the criteria described above. Six of the 28 patients who failed to respond to chlorpropamide were "brittle" type diabetics, requiring large doses of insulin and having a pronounced tendency to acidosis. For reasons such as fear of injections or recentness of onset of diabetes, 4 patients had not previously taken insulin. These cases were easily controlled with chlorpropamide. Three patients who had been taking insulin for more than 3 years remained well controlled after chlorpropamide was discontinued. However, one of these (FIGURE 1) developed hyperglycemia without glycosuria after 2 months without medication. This was relieved by a small daily dose of 0.25 gm. chlorpropamide. Only 2 of the 10 cases classified as "primary tolbutamide failures" responded well to chlorpropamide. One of these is shown graphically in FIGURE 2. Our experience with "secondary" or "late tolbutamide failures" was much better. Twenty of the 31 (64 per cent) patients in this group were managed satisfactorily with chlorpropamide. A representative case is shown in FIGURE 3. Another noteworthy feature was a good response to a second course of tolbutamide in 6 patients after chlorpropamide was stopped because of side effects (FIGURE 4). We suspect that the patients who responded to a second course of tolbutamide did so because of a renewed respect for adherence to diet.

Toxicity

Side reactions to chlorpropamide are listed in TABLE 2. Gastrointestinal symptoms of anorexia, nausea, vomiting, and abdominal discomfort were the most prominent. Chest pain severe enough to warrant cardiovascular investigation was noted in 8 patients. Electrocardiograms in all of these showed evidence of coronary artery disease and two of these later developed myocardial infarction. Chlorpropamide was continued in 2 of these cases and the chest pain gradually subsided. In fact, 2 patients were given chlorpropamide with good effect soon after suffering an acute myocardial infarct. Another prominent complaint was weakness, usually of legs, arms, and abdominal muscles. Two of the 7 patients with this complaint had

TABLE 2
SIDE REACTIONS TO CHLORPROPAMIDE

	No. of patients*
Anorexia, nausea, vomiting, abdominal discomfort.....	9
Chest pain.....	8
Muscle weakness.....	7
Hypoglycemic symptoms (anxiety, hunger, sweating).....	4
Rash and itching.....	4
Peculiar taste, numbness of tongue.....	3
Leukopenia (2500).....	1

* Of a total number of 70 patients treated.

responded as far as diabetic control was concerned, but the symptom was so severe that it became necessary to discontinue the drug. Serum potassium levels determined before and during treatment with chlorpropamide showed no diminution.

Hypoglycemic symptoms (anxiety, hunger, sweating) were noted in 4 patients. These never progressed to coma and disappeared promptly with administration of sweet drinks and reduced dosage.

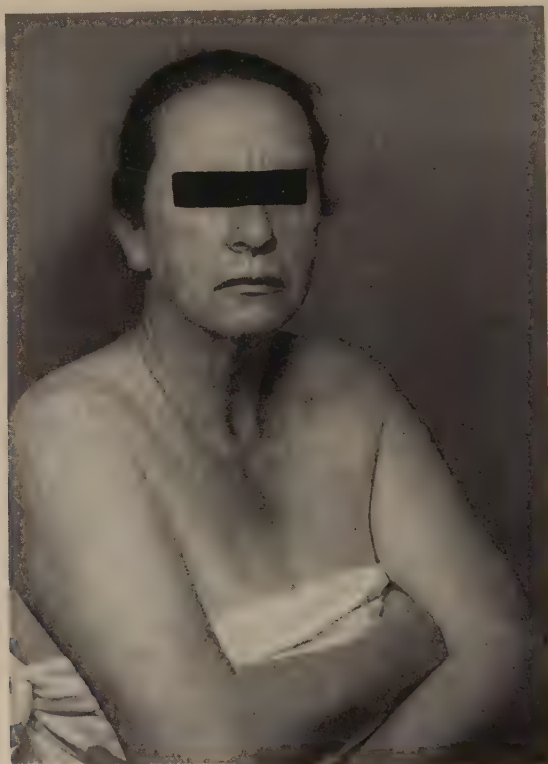


FIGURE 5. Maculopapular rash on face, neck, and arms following administration of chlorpropamide.

Four patients with itching, two with associated maculopapular rash of face, arms, and legs were noted. One of these (FIGURE 5) was severe enough to warrant discontinuing medication, but the others responded to antihistamine preparations without discontinuing chlorpropamide.

Three patients had an excellent response to a daily dose of 1.0 gm., but asked for a change of medication because of a disagreeable taste sensation. This was described as a "sick, sweet taste, even in the lips." In retrospect we believe this could have been avoided by starting with a smaller daily dose of 0.5 gm.

One case of leukopenia with a white blood cell count of 2500 was noted. This was not permanent; when medication was discontinued, the count

promptly returned to normal. The same patient complained of loss of hair after chlorpropamide had been discontinued. Examination failed to substantiate this disorder.

A depression of thyroid uptake of radioiodine was noted in 6 patients. The range of lowering was 4 to 15 per cent. There were no associated symptoms of hypothyroidism and this reaction was not considered of clinical significance.

No unusual effects on kidneys or liver have been noted to date. The longest period of observation has been 8 months.

One patient died of coronary thrombosis after having been controlled with chlorpropamide for 4 months. He was known to have malignant hypertension and his death was in no way attributable to the drug.

Conclusions

Of 70 diabetics studied, 60 per cent were controlled satisfactorily with chlorpropamide.

The most easily controlled were middle-aged patients with relatively recent onset of diabetes, requiring less than 40 U. of insulin for control.

Sixty-four per cent of patients who lost responsiveness to tolbutamide were managed satisfactorily with chlorpropamide.

Toxicity to chlorpropamide is related partly to dosage. Gastrointestinal symptoms, chest pain, and muscular weakness were noted in one-third of the patients studied. Occasionally these were relieved by reduction in dose of the drug.

In most cases a smaller dose of chlorpropamide gave as good if not better control than tolbutamide.

Unstable diabetics of any age are poor candidates for control with chlorpropamide.

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A LABORATORY AND CLINICAL STUDY OF CHLORPROPAMIDE IN AMBULATORY DIABETICS

Milton B. Handelsman, Leon Levitt, and Matthew F. Calabretta
*Diabetes Clinic, Long Island College Hospital, and Department of Medicine, State
University of New York Downstate Medical Center, Brooklyn, N. Y.*

Pharmacological studies have indicated that chlorpropamide is a potent hypoglycemic agent that is relatively nontoxic. With a background of this knowledge, clinical application of the drug was instituted in the Diabetes Clinic of the Long Island College Hospital 7 months ago. Six elderly mild diabetics were given 1 tablet (500 mg.) of chlorpropamide 3 times a day (a total of 1.5 gm.) on the first day and 1 tablet twice daily (a total of 1 gm.) thereafter. Four of the patients complained of severe muscle weakness and dizziness, leading to discontinuance of the drug. This initial experience prompted a more detailed investigation of the pharmacological properties of chlorpropamide in humans, since the experience obtained with other oral hypoglycemic agents did not seem to apply to this more potent agent.

A method of estimating chlorpropamide, as developed by the Research Department of Chas. Pfizer & Co., Inc., Brooklyn, N. Y., as described elsewhere in this monograph by Toolan and Wagner, was employed to study the level of the drug in sera of patients following single doses as well as during long-term administration of the medication. The results of these investigations are the subject of the present work. Clinical observations concerning the efficacy of the medication observed while treating ambulatory diabetics will be discussed elsewhere.

Serum Levels Following Single Doses Given Orally or Intravenously

Serial estimations of the serum level of chlorpropamide as well as blood sugar were made at 20-min. intervals for 4 hours after the administration of single doses of the medication. TABLE 1 shows a summary of the serum drug levels found after a 0.5-gm. dose was given orally and after an equivalent dose of the drug was given intravenously as the sodium salt. The intravenous injections were accomplished over a 2-min. period. Both diabetic and non-diabetic patients were studied. Two facts are to be noted. First, the drug was well absorbed, although somewhat slowly when given orally. Two hours after oral administration high serum levels were obtained, and within 4 hours the concentrations in serum were in a range similar to those obtained after the intravenous injection. Second, an appreciable quantity of the medication was still found in the serum 24 hours after its administration.

These observations concerning the good absorption and prolonged retention of the drug in serum were again found when 1-gm. doses of chlorpropamide were given orally and intravenously (TABLE 2, A and B). In this series oral administration was found to yield serum values at 4 and 24 hours that were higher than those obtained after a similar dose had been given intravenously.

This finding suggested that slow absorption from the intestinal tract might be a factor causing the prolonged elevation of drug levels in the blood. As can be seen in TABLE 2 (C and D), after giving single oral doses of 1.5 and 2 gm., proportionate increases in serum chlorpropamide levels were not obtained in 4 hours in most experiments. The larger doses led to higher concentrations in the serum after 24 hours.

TABLE 1
SERUM LEVELS OF CHLORPROPAMIDE AFTER SINGLE DOSE OF 0.5 GM.

Case	Diabetic Non- diabetic*	Body habitus†	Serum level (mg. %) at						
			20 min.	40 min.	1 hour	2 hours	3 hours	4 hours	24 hours
A—Intravenous									
C. H.	N.D.	O	7.9	6.7	6.3	5.6	5.8	5.0	3.1
J. S.	N.D.	O	4.5	4.0	4.8	4.0	3.6	3.8	1.6
J. G.	N.D.	T	6.4	6.8	7.2	6.2	5.6	5.6	2.4
B. S.	N.D.	T	7.9	6.3	7.0	6.0	6.0	5.8	3.0
C. H.	D.	M	7.2	7.0	5.7	6.4	5.7	2.2	—
D. H.	D.	O	3.3	4.3	4.3	6.1	6.2	5.3	4.7
G. R.	D.	O	7.2	4.5	4.3	—	5.9	4.3	0.3
D. T.	D.	M	6.9	6.2	5.6	5.2	5.6	5.1	1.9
Q. I.	D.	O	6.2	5.5	5.5	4.9	4.6	4.4	1.7
			Mean 6.39 S.D. 1.55	5.70 1.17	5.63 1.06	5.55 0.81	5.44 0.83	4.61 1.11	2.34 1.30
B—Orally									
J. Q.	N.D.	O	2.4	2.6	3.0	7.1	6.3	5.8	—
T. C.	N.D.	O	0	0.9	1.7	2.5	3.3	3.6	2.5
G. P.	D.	M	0	1.8	2.2	2.5	—	4.4	2.5
L. F.	D.	M	0	1.0	1.4	4.8	5.7	5.0	2.0
			Mean 0.6 S.D. 1.08	1.58 .79	2.08 .70	4.23 2.20	5.10 1.59	4.70 0.93	2.33 0.28

* Symbols: D = diabetic; N.D. = nondiabetic.

† Symbols: O = obese; T = thin; M = medium.

The variations in blood levels obtained in different patients after the same dose of chlorpropamide had been given (TABLES 1 and 2) could not be related to differences in absorption, since it occurred after intravenous administration as well. The size of the patient influenced this to some extent. In TABLE 2, A, patients M. V. and R. A. were large and obese; after receiving 1 gm. of the drug they had serum drug levels that were similar to those obtained when 0.5 gm. was given to patients of normal size. However, no constant relation to body weight was found when calculations were made of the distribution of the drug, assuming that the medication remained in the extracellular space. In obese patients the space was usually larger (25 to 27 per cent) and in thin patients such calculations found it usually smaller (16 to 18 per cent).

TABLE 2
SERUM LEVELS OF CHLORPROPAMIDE AFTER SINGLE DOSES

Case	Diabetic Non- diabetic*	Body habitus†	Serum level (mg. %) at						
			20 min.	40 min.	1 hour	2 hours	3 hours	4 hours	24 hours
A—Intravenous—1.0 gm.									
M. V.	N.D.	O	4.5	5.4	4.5	5.1	—	—	—
T. F.	N.D.	T	14.5	13.1	12.0	11.7	11.4	10.3	—
R. A.	N.D.	O	5.3	5.4	5.4	4.8	4.6	3.2	2.4
F. C.	D.	T	6.4	13.7	10.5	10.3	9.8	9.8	3.6
W. N.	D.	T	12.5	10.2	10.6	8.9	—	8.5	1.3
Mean			8.6	9.6	8.6	8.2	8.6	8.0	2.4
B—Orally—1.0 gm.									
S. B.	N.D.	T	3.6	8.1	10.1	12.9	14.8	13.9	4.8
A. M.‡	N.D.	T	0	0	1.4	7.9	13.2	14.0	9.1
F. M.‡	D.	O	0.2	6.1	8.9	13.0	15.1	12.1	9.4
T. W.	D.	M	3.3	7.8	8.0	9.9	10.5	8.8	6.9
Mean			1.8	5.5	7.1	10.9	13.4	12.2	7.6
C—Orally—1.5 gm.									
M. G.	N.D.	M	3.8	5.1	6.1	15.9	18.1	19.7	13.0
F. G.	N.D.	M	2.5	2.9	3.6	7.4	13.8	15.8	9.3
F. D.	D.	T	1.0	5.2	8.4	14.5	12.3	13.2	4.4
M. K.	D.	M	3.0	3.6					
Mean			2.6	4.2	6.0	12.6	14.7	16.2	8.9
D—Orally—2.0 gm.									
S. T.	N.D.	T	—	—	22.4	—	—	21.4	16.7
J. N.	N.D.	M	4.4	8.4	9.2	21.5	15.6	15.2	15.8
A. S.	D.	O	3.9	5.1	7.1	12.2	13.4	13.0	6.0
Mean			4.2	6.8	13.4	16.9	14.5	16.5	12.8

* Symbols: D = diabetic; N.D. = nondiabetic.

† Symbols: O = obese; T = thin; M = medium.

‡ In 48 hours A.M. had a chlorpropamide level of 3.7 mg. % and F.M., 5.4 mg. %.

Serum Levels of Chlorpropamide After Continued Drug Administration

In view of the prolonged retention of the drug after a single dose, serum levels were studied in patients receiving continued daily doses. The amount prescribed was conservative in view of the previously mentioned experience in which 1 or 1.5 gm./day led to toxic symptoms. In general, 1 tablet of 500 gm. was given as a single daily dose. After 1 or 2 weeks the dosage was cut to 250 mg./day in older and very thin patients. In some instances, the latter type of patients were given 250 mg./day from the outset. Since one

of the important purposes of this investigation was to ascertain possible cumulative effects after continued administration, frequent changes in dosage were avoided.

The above treatment was prescribed for 52 ambulatory diabetic patients who were given the medication from 6 to 26 weeks. Serial estimations of chlorpropamide concentrations were made of the blood, which was also analyzed for glucose content and hepatic and renal function profiles. The value of checking blood concentrations became apparent quite early. Four

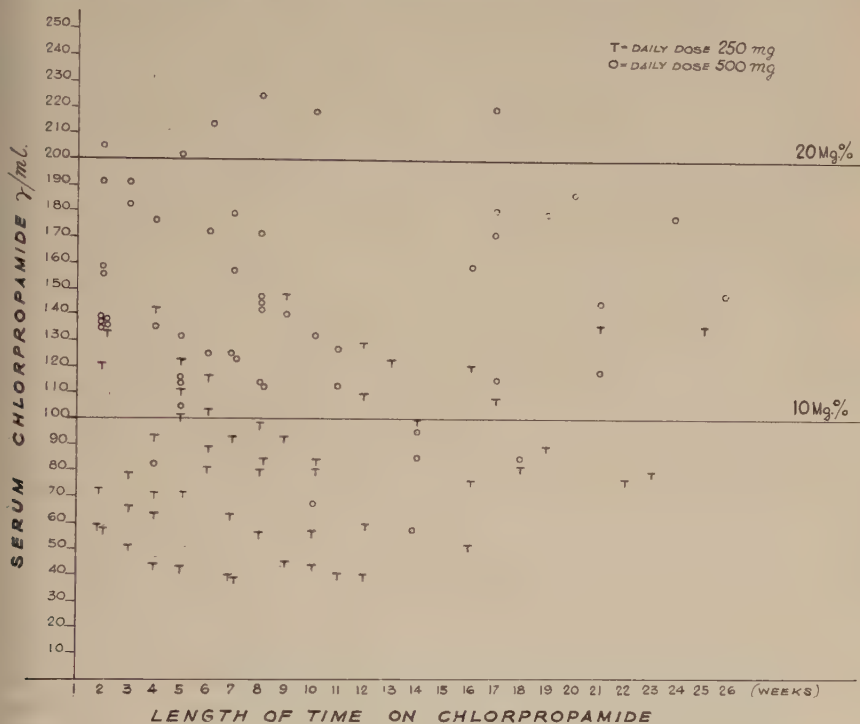


FIGURE 1. Serum chlorpropamide levels after prolonged administration.

patients reported to the clinic regularly and insisted that they took the medication, yet zero levels of chlorpropamide were found in the blood. Two confessed later that they had not, but the other two individuals insisted to the end that they had taken the medication. Twenty-two patients took 500 mg./day, and 25 others took 250 mg./day.

In FIGURE 1 the blood levels of chlorpropamide are plotted against the length of time the drug was taken. In the patients taking 250 mg., most points fall between 4 and 10 mg. per cent. This range is lower than that found in a control group of normal patients given the same dose, as reported elsewhere in this monograph by Carlozzi and Iezzoni. The lower range might well be explained by patients' omission of occasional doses. There were 2 instances in which the blood levels were consistently elevated; it is

possible that these patients took aspirin, which interferes with the chemical estimation of chlorpropamide.

With a daily dose of 500 mg. the scatter was much greater. Again, 2 patients showed unexpectedly low values. A history of omission of doses could not be obtained in these patients, but the trustworthiness of such a history cannot be guaranteed. Serum chlorpropamide levels ranged from 11 to 22 mg. per cent. In general, the lower values were found in the obese patients and higher values in small, thin patients, but this could not be correlated statistically.

An observation constantly noted was that patients who had low serum drug levels on a given daily dose continued to have relatively low concentrations while receiving the same dose. The same finding occurred in patients who showed high serum levels in the first 2 or 4 weeks; they continued to have the higher concentrations for months if the dose was left unchanged. Thus, in the doses used, the blood level tended to be characteristic of the patient. After a plateau level was attained, a cumulative effect was not noted.

Relationship Between Drug Levels and the Hypoglycemic Effect After Single Doses

Tests performed by estimating simultaneously the venous blood sugar levels and serum drug concentrations allowed the relationship between the 2 to be studied. The former declined at a slow continuous rate while the latter rose gradually. FIGURE 2 shows representative experiments in diabetics and nondiabetics. After 0.5 gm. was administered orally, the blood sugar began to fall when the chlorpropamide concentration was between 2 and 3 mg. per cent (FIGURE 2, T. C.). The drop was maintained for 4 hours, when the drug levels were no higher than 6 mg. per cent (FIGURE 2, L. F.). The hypoglycemic potency of chlorpropamide at these low dosage levels posed the question as to what effect larger doses might have. When 2.0 gm. were given orally and the drug was slowly absorbed (FIGURE 2, A. S.), the blood sugar also dropped slowly. When absorption was rapid, leading to initially high serum levels, the drop in blood sugar was rapid (FIGURE 2, J. T.).

In practically all the oral experiments, the blood sugars continued to decline throughout the entire 4-hour period. This was found in both nondiabetics and diabetic "responders." With the intravenous administration of the drug, however, nondiabetics showed a greater initial drop, after which the blood sugar tended to rise even when the drug blood levels were 10 and 12 mg. per cent. In some cases this rise in blood sugar in the face of an adequate drug level was sufficiently marked to lead one to suspect that drug concentration in the liver or other organs might be more significant than that in the systemic blood.

Antidiabetic Action of Chlorpropamide

Many studies have reported an inability to correlate the single dose response of various oral hypoglycemic agents with their therapeutic efficacy. The blood sugar, although important, is in many respects only an indirect

indicator of the complex and subtle alterations of metabolism leading to the well-known complications that occur in a diabetic organism. Demonstration of the hypoglycemic activity of a drug does not necessarily denote that it is antidiabetic. Even exogenous insulin was under suspicion by the late H. Mosenthal, who stated: "Insulin, administered by injection, is not the complete remedy for diabetes, because the greater part of the injected insulin acts in the extrahepatic tissues and results in the deposit of glycogen in

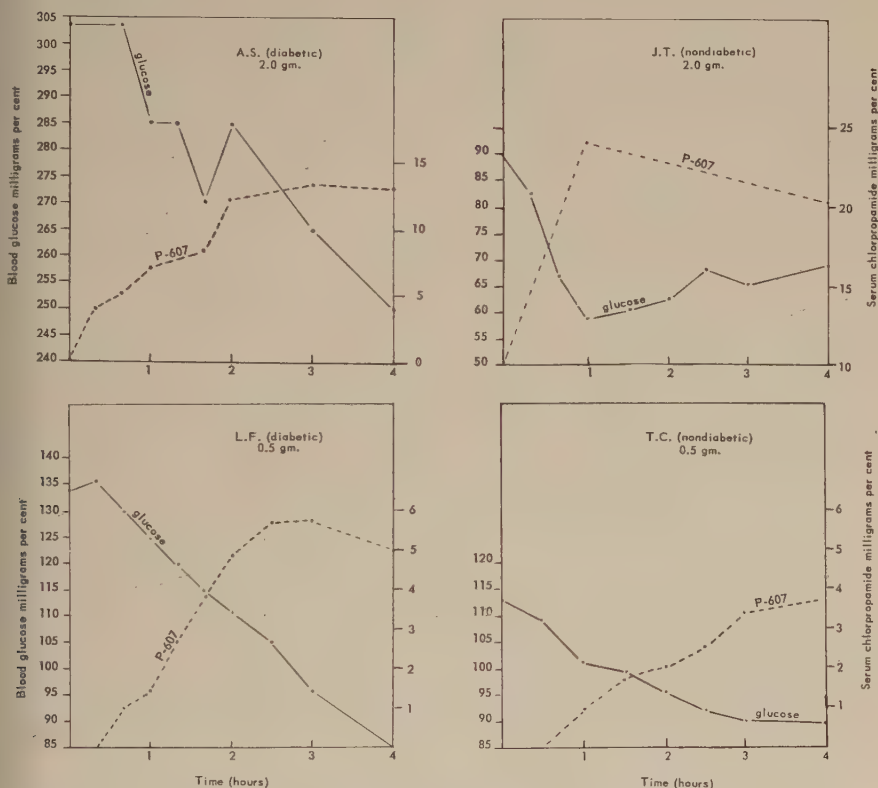


FIGURE 2. Blood sugar fall and chlorpropamide levels after a single dose of drug.

them."¹ In this light, a total evaluation of the antidiabetic properties of chlorpropamide must await many years of clinical observation. However, any attempt to initiate such an evaluation can begin by employing human diabetics of the maturity-onset type as "test objects," since such patients are known to respond to the drug.

Thirty-five of the 47 ambulatory patients who were selected for treatment with chlorpropamide were of this type and had previously been followed in the Diabetes Clinic for at least 6 months on other treatment. The aim of treatment in this clinic is to obtain a physiological level of control and optimal nutrition. The present group of patients was selected either because they

did not attain these criteria, or because a comparison was desired between chlorpropamide and previous treatment. The patients kept charts of their urine tests (Clinitest) before meals, and frequent fasting blood sugars were obtained.

The majority of our patients speak only Spanish, Italian, or Arabic, and live in families where the native dishes are chiefly of high carbohydrate content; obesity is common. In TABLE 3 (Group A) are assembled 15 uncomplicated obese mature diabetics of recent onset. According to past observations of Duncan² and of this clinic³ probably all could be excellently controlled by weight reduction with a resultant increase in glucose tolerance. Varying degrees of lack of cooperation in dieting were the cause of poor diabetic control, and all in Group A had not lost weight while presumably dieting. In the first 4 cases the addition of chlorpropamide was definitely of value, obtaining a level of control that was not obtainable in the previous period. The fifth patient demonstrated that chlorpropamide does not allow unlimited eating of cake; she refused insulin therapy.

The next 7 patients had received insulin for at least 1 year prior to this study. Replacement of the insulin with chlorpropamide led to better, or at least equivalent, results in all but 1 patient (N. O. C.). Many of these patients volunteered the information that they previously had frequent mild attacks of hunger that had forced them to eat more, and that they felt better without insulin. Despite these allegations, weight loss was uncommon even when on chlorpropamide.

The last 4 patients had previously received tolbutamide. With the exception of patient N. O. C., who did badly with all attempts at treatment, 0.25 gm. of chlorpropamide seemed to do as much as 1 gm. of tolbutamide. Control of these patients might have been better if larger doses of chlorpropamide had been used.

In TABLE 3, Group B is composed of a similar type of obese adults with diabetes of long standing. They had received insulin for 9 or more years and had remained obese. The replacement of the insulin with chlorpropamide led to better control in half these patients. Only 2 patients had previously been given tolbutamide; chlorpropamide in 0.5-gm. doses appeared to be slightly superior to 1.5 and 2 gm. of the former drug.

Diabetics who are thin pose a different problem. Adequate nutrition must be given and thus, from the onset, their diets contain a relatively large amount of carbohydrate. TABLE 4 shows the results with thin adult diabetics; Group C contains 3 patients who were always thin, developing diabetes later in life. This number was small but seemed to show the value of the oral hypoglycemic agent in obtaining a better level of control while such patients were on a diet high in carbohydrates and in calories. A larger number of thin diabetics were obese at the time the diabetes manifested itself and are shown in Groups D and E. In Group D the majority of the patients had lost weight before coming to the clinic, usually due to neglect in treatment, a recent serious illness, or previously undiagnosed diabetes. Insulin was usually prescribed when the patients first presented themselves, to allow an ample diet to be given. When chlorpropamide was given a gradual reduction of

MATURITY-ONSET TYPE OF DIABETES: OBESE PATIENTS

Group A: relatively recent onset; no weight loss

Patient	Age	Duration (years)	Insulin (units) daily	Urine glucose (per cent)	Blood glucose (mg. %)	Tolb.* (gm.) daily	Urine glucose (per cent)	Blood glucose (mg. %)	P 607† (gm.) daily	Urine glucose (per cent)	Blood glucose (mg. %)
P. U. G.	51	1	0	0	186-238	—	—	—	0.5	0	116-140†
M. O. L.	40	1	0	0	162-197	—	—	—	0.25	0	96-130†
P. I. A. Z.	42	1	0	1-2	158-213	—	—	—	0.25	0	96-120†
N. A. D.	68	8	0	0	200-266§	—	—	—	0.25	0	140-144
S. A. V.	50	3	0	2	273-348§	—	—	—	0.5	0-1/4	225-259§
L. O. R.	61	1	20 PZI	0-2	144-155	—	—	—	0.5	0-1/4	136-155
O. N. A.	53	5	15 NPH	1/2-2	149-197	—	—	—	0.25	0	96-140†
V. E. G.	64	1	20 NPH	0	116-140†	—	—	—	0.25	0	73-116†
Z. A. R.	67	3	15 NPH	0	160-202	—	—	—	0.25	0	102-138†
M. O. N.	71	2	25 NPH	0	171-191	—	—	—	0.25	0	181-184
K. O. E.	65	5	38 NPH	0	181-202	—	—	—	0.25	0	165-176
N. O. C.	65	4	35 NPH	0-1/4	223-260§	3	0-1/4	181-298§	0.75	0-1	202-266§
H. A. Y.	46	6	0	0	244-250§	1 1/2	0	108-113†	0.25	0	130-208
V. I. T.	50	3	0	2	290-307§	1	0-3/4	158-176	0.25	0	162-181
A. Y. E.	71	7	0	0-2	156-180	1	0-2	158-181	0.25	0	178-197

Group B: Obese at onset; long-standing diabetes; still obese

Patient	Age	Duration (years)	Insulin (units) daily	Urine glucose (per cent)	Blood glucose (mg. %)	Tolb.* (gm.) daily	Urine glucose (per cent)	Blood glucose (mg. %)	P 607† (gm.) daily	Urine glucose (per cent)	Blood glucose (mg. %)
K. A. R.	59	11	0	0	190-240	1 (BZ 55)	0	160-161	0.25	0	113-140†
L. O. V.	67	11	35 NPH	0-2	220-264§	2	0-tr	268-398§	0.5	0	176-197
D. E. L.	67	16	25 NPH	0-2	260-318§	1.5	1/4-3/4	360-396§	0.5	0	245-273§
F. O. N.	56	11	35 Lente	0-1	162-298§	—	—	—	0.5	0	232-316§
M. I. L.	61	9	12 NPH	0-2	197-232§	—	—	—	0.5	0-tr	113-148†
S. P. F.	57	21	20 PZI	0-2	218-219§	—	—	—	0.25	0	109-144†

* Tolbutamide.

† Chlorpropamide.

‡ Excellent control: urines always free of glucose; fasting blood sugar 80 to 120 mg. per cent at most times.

§ Bad control: urines usually contain sugar; fasting blood sugar usually over 250 mg. per cent.

The maximum and minimum urine and blood sugars during the period of observation are given.

TABLE 4
MATURITY-ONSET TYPE OF DIABETES: THIN PATIENTS

Patient	Age	Duration (years)	Insulin (units) daily	Group C: always thin			P 607† (gm.) daily	Urine glucose (per cent)	Blood glucose (mg. %)
				Urine glucose (per cent)	Tolb.* (gm.) daily	Urine glucose (per cent)			
Q. U. L.	65	8	—	0	—	—	0.25	0	150-197
M. A. R.	84	2	—	0-tr	0.5	0	0.25	0	144-158
C. A. S.	48	1	—	—	2	0	0.25	0	120-132‡
Group D: thin; obese at onset; recent diabetes									
F. A. V.	71	4	65 NPH	0	—	—	20 NPH 0.5	0-1/4	167-202
K. L. E.	68	2	35 PZI 20 PZI	0-1	—	—	0.5	0-tr	137-161
M. I. R.	65	3	15 reg. 15 NPH	0	—	—	0.25	0	166-213
T. O. R.	76	2	—	0-tr	—	—	0.5	3/4-1	202-281§
Group E: thin; obese at onset; long-standing diabetes									
Z. I. S.	64	14	24 NPH	0	—	—	0.5	0	124-165
S. A. D.	73	18	25 PZI	0-1	—	—	0.5	0	130-176
F. L. O.	69	12	15 Lente	1-2	—	—	0.5	0	166-184
F. R. A. N.	65	25	25 Globin	0-2	—	—	0.5	1-2	195-232§
S. C. H.	73	21	25 NPH	0	6	0-2	10 NPH 0.25	0	166-171
S. U. N.	69	21	0	0	1.5	0	0.5	0	102-106‡
B. O. D.	74	16	0	0-1/4	—	—	0.25	0	225-232§

* Tolbutamide.

† Chlorpropamide.

‡ Excellent control.

§ Bad control.

insulin was made in patients receiving 25 units or more. The insulin was abruptly stopped in those taking less.

It should be noted that the thin cases of recent onset (Group D) responded similarly to those of long-standing diabetes (Group E). At the time that therapy with chlorpropamide was initiated, the level of control in these 11 patients (both groups) was excellent in only 1 patient and fair in only 2, even while on insulin. Subsequently, on oral therapy, after discontinuing insulin, 5 patients (K. L. E., M. I. R., Z. I. S., S. A. D., and F. L. O.) of the 11 demonstrated diabetic control that was equivalent to or better than when on insulin. Two cases who originally were badly controlled were markedly improved. One patient (S. C. H.) who was completely unresponsive to 6 gm. tolbutamide/day obtained fair control with 10 U. NPH and 0.25 gm. chlorpropamide. The best result in this group was obtained in a cooperative patient (S. U. N.), who attained a physiological level of control and gained weight with 0.5 gm. chlorpropamide but could not do so with diet alone or with 1.5 gm. tolbutamide. This report represents a preliminary, and therefore incomplete, study. Out of the total of 35 patients in TABLES 3 and 4, there were 7 primary failures. There is reason to believe that in many of our patients further manipulation of the dosage schedule might lead to better results.

It is important to reemphasize clinical results in treatment. The above charts giving urine and blood sugar levels record only one of many criteria that should be used in assessing the antidiabetic quality of a medication. In our obese diabetics the mere prescribing of a diet without adequate cooperation by the patient had failed, and many had such complaints as pruritis vulva and thirst alleviated by the oral hypoglycemic agent. In many thin adult patients chlorpropamide allowed better utilization of the ingested food, leading to desired gains in weight. Such results can only be inferred from charts but, again, should be mentioned.

Toxicity

To study the incidence of toxicity, the entire series of 47 ambulatory patients was reviewed. It has already been stated that, with a daily dosage of 1.5 gm., 4 out of 6 patients had severe asthenia, muscular weakness, and lightheadedness. In the blood of one of these patients there was a drug level of 46.6 mg. per cent and a blood sugar of 225 mg. per cent after 2 weeks of medication. Such incidences of toxicity must be attributed to overdosage rather than to the drug itself. Recurrence of severe diabetic symptomatology occurred in 2 young adults with severe diabetes in whom the insulin was reduced when chlorpropamide was given. The latter should be attributed to "nonresponsiveness" rather than toxicity. Two patients without previous gastrointestinal complaints developed acute cholecystitis, confirmed by surgery, 1 and 2 months after taking chlorpropamide. These were probably coincidental.

In all, 2 patients had pruritis while on therapeutic doses, requiring discontinuance of the drug, and 2 patients had dizziness and nausea. One of the patients with pruritis previously had a pruritic rash when taking tolbut-

amide (Orinase). All symptoms quickly disappeared with discontinuance of the medication. Serial hematological surveys on all patients have revealed no anemia; a slight leukocytosis (10 to 11 thousand), with a normal differential leukocyte count, was frequently encountered without adequate explanation. Serial liver profiles also did not change. In this connection it should be mentioned that 3 diabetic patients with known hepatic cirrhosis have been given chlorpropamide. Liver biopsies had established the diagnosis in all, but 2 had normal liver function tests, and only 1 was active, as shown by a markedly elevated serum transaminase. The latter patient returned to normal while on chlorpropamide therapy. The liver profile in the other 2 patients remained normal.

The most serious manifestation of toxicity was encountered in a 56-year-old man who previously had urticaria when given Orinase. Immediately after the intravenous administration of 1 gm. chlorpropamide, he went into a state of shock that responded to an intramuscular injection of Chlor-Trimeton. Blood sugars prior to and during the episode were 167 and 165 mg. per cent., respectively.

Summary

Chlorpropamide is an extremely potent hypoglycemic agent that is quickly absorbed and slowly excreted. Oral administration in therapeutic doses leads to predictable drug levels in the serum. It has demonstrated favorable antidiabetic activity during the period of study (6 months) and can effectively replace insulin in diabetics with maturity onset. With it a better control can be obtained in that large segment of the diabetic population that does not fully cooperate in the dietary management. Dosagewise it is more potent than Orinase. Toxicity in therapeutic doses did not constitute a problem.

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CLINICAL EXPERIENCE WITH CHLORPROPAMIDE*

Barnett Greenhouse

Grace-New Haven Community Hospital, New Haven, Conn.

Chlorpropamide (P 607, Diabinese), a sulfonylurea compound and close chemical relative of tolbutamide (Orinase), has recently been introduced in the oral hypoglycemic field. A preliminary clinical study of the drug was begun early in January 1958 to determine its usefulness and safety as an oral medication for diabetes.

Clinical Pilot Study

Chlorpropamide was subjected to a clinical trial in 50 nonhospitalized diabetic patients ranging in age from 18 to 87, with an onset age of 18 to 72 years and a duration of the disease from 1 day to 33 years. Two-thirds of the patients studied have been on insulin for various periods to 33 years, and one-third of these were on dosages of 50 to 150 U.

The patients were under close clinical observation and controlled by frequent laboratory tests designed to disclose any toxic effects on the liver, kidneys and blood-forming organs. Laboratory profiles including blood sugar, urinalysis, peripheral hematogram, alkaline phosphatase, thymol turbidity and flocculation, and blood urea nitrogen were done at weekly, biweekly, and monthly intervals.

The compound was used in three basic clinical situations: (1) transfer from Orinase, (2) conversion from insulin, and (3) in newly discovered or previously untreated cases.

Transfer from Orinase to chlorpropamide. In the initial test series, Orinase-controlled diabetics (twenty-two patients) were shifted to chlorpropamide for comparative evaluation on a gram-for-gram basis, an 0.5-gm. tablet of chlorpropamide replacing an 0.5-gm. Orinase tablet. Control of the diabetes proved adequate, but various complications appeared; one gram of chlorpropamide per day produced multiple, bizarre, and at times disturbing changes in one-third of the patients. The reactions fell into the general category of side effects of the hypoglycemic "sulfas" with varying degrees of intensity. The subjective complaints were predominantly of a hypoglycemic nature with CNS, circulatory, and renal manifestations. Other side effects noted were gastrointestinal disturbances and allergic skin reactions.

There was no doubt that chlorpropamide lowered the blood sugar; the response was prompt and dramatic, exhibiting a greater potency than Orinase.

The side effects were always reversible and gradually subsided when dosage was lowered or the drug discontinued. Better tolerance was achieved generally, however, with a reduction in dosage, although some patients tolerated high dosages without ill effects.

A 200-mg. tablet of chlorpropamide was subsequently made available;

* The investigation reported here was supported by a research grant from Chas. Pfizer & Co., Inc., Brooklyn, N. Y.

with a lowering of the daily dose level below 500 mg., there was a decided improvement in clinical usefulness. Patients were successfully managed on the lower dosages with smooth control of hyperglycemia and glycosuria and with a low incidence of side reactions. Transfers from Orinase to chlorpropamide were subsequently made by replacing a 500-mg. Orinase tablet with a 200-mg. tablet of chlorpropamide. The medication was given in divided doses immediately after meals to minimize side reactions and to avoid gastric irritation.

Chlorpropamide was also found to restore control where Orinase effect declined or failed on long use (six patients). One insulin patient who did not respond to Orinase responded readily to chlorpropamide and discontinued insulin.

Conversion from insulin to chlorpropamide. Generally, where Orinase was known to be primarily ineffective, chlorpropamide was also found to be

TABLE 1
DURATION OF CHLORPROPAMIDE THERAPY IN PATIENTS WITH DIABETES MELLITUS

Duration, months	Number of cases			
	Total	Still on therapy	Lost to follow-up	Therapy discontinued
1	8	—	—	8
2	8	3	—	5
3	10	8	—	2
4	2	2	—	—
5	5	2	—	3
6	1	1	—	—
7	7	5	—	2
8	9	8	—	1
Total	50	29		21

without effect in therapeutic dosages (six patients). Larger doses of chlorpropamide, however, tended to reduce the insulin dose in some resistant cases (four patients).

In those cases previously treated with insulin but not with Orinase, conversion to chlorpropamide was possible without difficulty in 8 of 12 patients with an insulin range of 10 to 125 U.

Administration and dosage. Chlorpropamide was initiated by administering a 200-mg. tablet twice daily with a concurrent 20 to 50 per cent reduction in insulin; as soon as a satisfactory response was observed, insulin was slowly withdrawn. If the patient seemed unresponsive, an additional tablet was added every 3 to 5 days, but the total dosage seldom exceeded 1.0 gm. per day. At the same time the insulin was raised 2 to 4 U. daily as needed to combat glycosuria.

TABLE 2
CLINICAL TOLERANCE FOR CHLORPROPAMIDE: SIDE EFFECTS*

Hypoglycemia (14)

CNS (11): agitation, blurred vision, confusion, dizziness, depression, dullness, drowsiness, fatigue, flushing, grogginess, headache, hunger, incoordination, irritability, lack of well-being, lethargy, loss of concentration, malaise, nervousness, restlessness, sleeplessness, unsteadiness, weakness.

Circulatory (2): congestive failure, dyspnea, palpitation.

Renal (1): albuminuria, dyspnea, peripheral edema.

Allergic Skin Reactions (4)

Erythema Multiforme (1), pruritis (2), urticaria (1).

Gastrointestinal disturbance (12)

Anorexia, constipation, diarrhea, distention, flatulence, halitosis, heartburn, nausea, vomiting.

Alcohol intolerance (1)

Crystalluria ? (2)

Stippling of red blood cells (1)

* Figure in parentheses gives number of cases.

Actually, the dosages of insulin and chlorpropamide were chosen arbitrarily, and the patient was kept on the same dose of chlorpropamide as long as it proved effective and caused a decline in the insulin requirement. Meanwhile, without anticipating the effect of the drug, a close watch was kept on the insulin need to keep the condition from getting out of control or to prevent insulin shock. The regimen lent itself readily to outpatient and office management, and none of the patients required hospitalization.

New and untreated diabetics. Previously untreated and uncomplicated mild cases (4 patients) and a recently discovered adult diabetic patient responded promptly to chlorpropamide. As a routine, a 200-mg. tablet was given once or twice daily until the patient was sugar-free; then he was maintained on 1 tablet every morning after breakfast. Thereafter a second

DISTRIBUTION OF PATIENTS AND RESULTS OF
CHLORPROPAMIDE THERAPY IN 50 ADULT DIABETICS

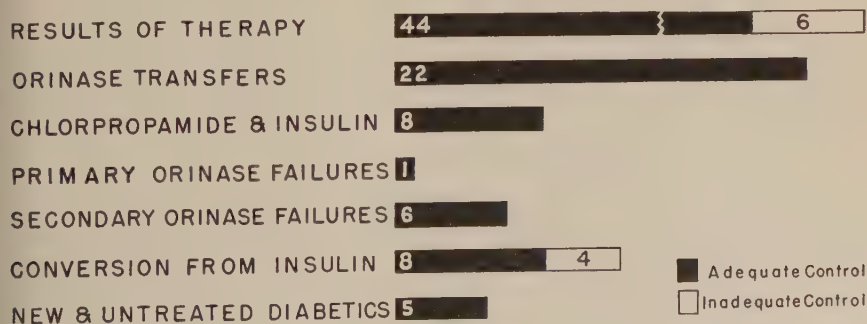


FIGURE 1

CORRELATION OF AGE AT TIME OF TREATMENT WITH RESPONSE TO CHLORPROPAMIDE

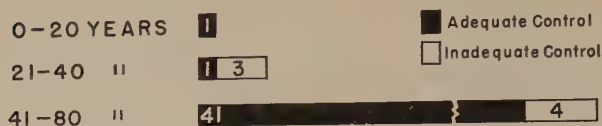


FIGURE 2

CORRELATION OF DURATION OF DIABETES WITH RESPONSE TO CHLORPROPAMIDE

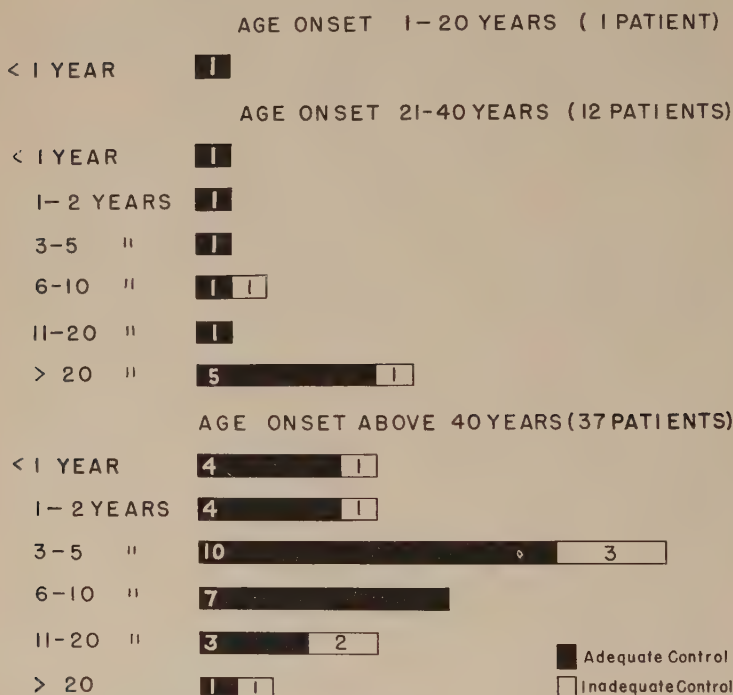


FIGURE 3

tablet was allowed after supper whenever the dinner test was positive for sugar.

Toxicity. Excluding toxic drug reactions inherent in overdosage, no persistent toxicity was encountered at therapeutic levels. About the only evidence of toxicity, which relates to other sulfonamides as well, has been the development of some skin rashes and itching (four patients). Otherwise, there was no evidence of toxicity that could be determined by studies of liver function, renal function, and the blood elements. No acetonuria was encountered in the responsive patients. Dulling of the appetite was noted by some.

CORRELATION OF DURATION OF DIABETES WITH RESPONSE TO CHLORPROPAMIDE

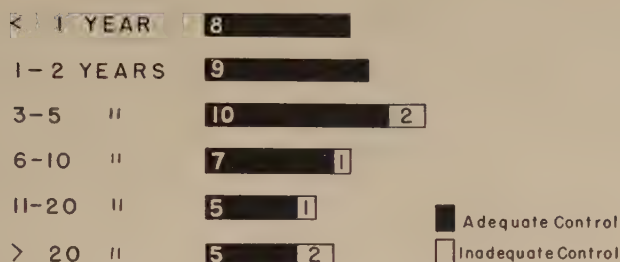


FIGURE 4

CORRELATION OF PRIOR INSULIN DOSE WITH RESPONSE TO CHLORPROPAMIDE

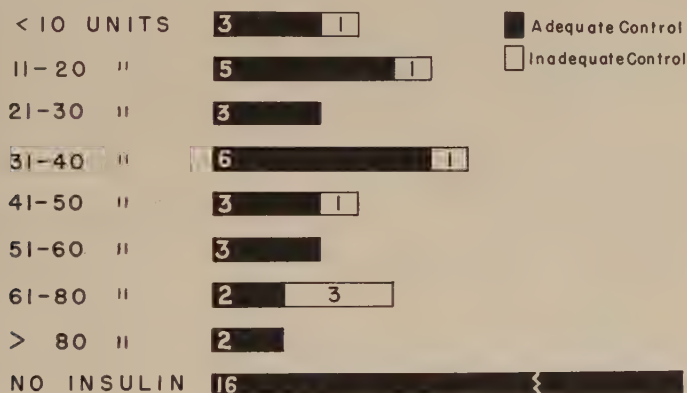


FIGURE 5

DAILY DOSAGE OF CHLORPROPAMIDE REQUIRED FOR MAINTENANCE CONTROL

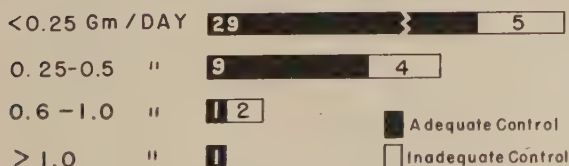


FIGURE 6

EXPERIENCE WITH CHLORPROPAMIDE THERAPY DISCONTINUED

NO. OF PATIENTS	50
UNDESIRABLE SIDE EFFECTS	6
SUGAR-FREE	9
INEFFECTIVE	6

FIGURE 7

Clinical Results. One half of the group of 50 patients studied has been under continuous treatment for 3 to 8 months (TABLE 1); approximately one-fourth of the patients were eliminated in the first 2 months because of side effects (TABLE 2) or lack of response to chlorpropamide. Adequate control was obtained in 44 patients, of whom 8 were maintained on chlorpropamide and insulin; results were inadequate in 6 (FIGURE 1).

Chlorpropamide was found to be effective chiefly in patients more than 40 years old (FIGURE 2) whose age at onset of diabetes was more than 20 years and, in particular, in those older than 40 at onset (FIGURE 3). The duration of the disease was not significant, the drug showing good effect in patients who have had diabetes for as long as 25 years (FIGURE 4). Patients on prior insulin dosages of less than 50 U. showed a good response, although equally good response was had in several patients with an insulin range of 50 to 125 U. (FIGURE 5). Daily dosage required for maintaining control ranged between 100 and 400 mg. (FIGURE 6).

The drug was stopped in nine patients who became sugar-free; it was ineffective in six patients, and six patients abandoned medication because of side effects (FIGURE 7). Abnormal tolerance (TABLE 3) of varying degrees occurred most frequently on higher dosages in twenty patients, nearly one-half of whom showed multiple side effects.

TABLE 3
CORRELATION OF DAILY DOSAGES OF CHLORPROPAMIDE WITH NUMBER OF
SIDE EFFECTS

Side effect	Grams per day			
	<0.25	0.5	1.0	>1.0
Hypoglycemia and sequelae.....			14	
Gastrointestinal.....	1		11	
Skin.....		1	2	1
Alcohol intolerance.....		1		
Red blood cell stippling.....	1			
Crystalluria (?).....	2			

Glycosuria and elevated blood sugars recurred after two to six months in three patients who had been on chlorpropamide therapy; one of these was previously shifted to chlorpropamide because of secondary failure on Orinase.

Comment

Preliminary and early use of chlorpropamide was undertaken without previous knowledge of the clinical effects of the drug. During the exploratory phase, guided by experience with Orinase, chlorpropamide was first administered, dose for dose, to a selected group of Orinase-controlled patients for comparative evaluation. The response was prompt and unquestionable. Chlorpropamide proved to be a potent and rapidly active hypoglycemic agent with an activity more than twice that of tolbutamide. Doses in the magnitude of 1.0 gm. were found to induce adverse side effects that, for the most part, were correlated with hypoglycemic manifestations and sequelae characteristic of the long-acting insulins. Expanding trials, however, showed chlorpropamide to be generally well tolerated and clinically effective in the therapeutic range of 100 to 400 mg. and to have no significant undesirable by-effects.

In view of its enhanced potency and rapid action as observed clinically, treatment with chlorpropamide was initiated without a priming dose. In the newly treated diabetic the response to the drug was found to be rapid with a minimum dose from the start; for conversion from insulin, a basic dose of two 200-mg. tablets was quickly followed by a reduction in insulin requirement; and for transfer from Orinase, a 200-mg. tablet of chlorpropamide replaced a 500-mg. Orinase tablet with comparable clinical effect.

Indications for chlorpropamide are the same as those for Orinase; stable or relatively mild diabetics of the middle-age or older group respond best to the drug. The responsiveness of the early case is very striking. The mild diabetics and, by that token, the older patients, are very sensitive to the drug. Neither duration of the disease nor prior insulin dosage appear to be significant. Because of its evidently greater hypoglycemic activity, chlorpropamide may be found effective even beyond the limits of Orinase in dosages tolerated beyond the therapeutic range, and it may thus prove to be a truer measure of drug responsiveness than Orinase.

A significant observation involves the effectiveness of chlorpropamide in primary and secondary Orinase failure. The development of a refractory tolerance to Orinase, experienced on prolonged use of the drug, is becoming evident also with chlorpropamide. Stopping medication at intervals to allow for a rest period, and starting again when and if glycosuria recurs are measures aimed at preventing drug resistance.

Chlorpropamide and Orinase have been used interchangeably and, currently, a shift from one to the other also is being explored as a possible means of avoiding an acquired tolerance to either.

Hypoglycemic reactions on small doses (200 mg.) of chlorpropamide have been observed in sensitive individuals, indicating the need for adjusting the maintenance dose to the lowest level consistent with good metabolic control.

The management of diabetes with chlorpropamide is smooth and devoid

of the marked blood sugar fluctuations and wide metabolic excursions characteristic of insulin. Patients feel better and are less agitated, and excessive appetite is curbed by the anorexic effect of the drug, as well as by the normalization of the blood sugar. Absence of acetonuria in the chlorpropamide-responsive patients reflects a favorable effect on carbohydrate metabolism with a stabilizing influence on the diabetic state. See case reports below.

Summary

Chlorpropamide (P 607, Diabinese), a new sulfonylurea derivative with a hypoglycemic activity similar to that of Orinase, but with enhanced potency and longer duration of action, is currently under clinical investigation.

An early pilot study and expanding trials with this compound give promise of its therapeutic value in the treatment of diabetes. It is proving to be an active and clinically effective oral hypoglycemic agent more than twice as active as tolbutamide. In the therapeutic range of 100 to 400 mg., it appears to be safe, effective, and well tolerated, with minimal side reactions.

Acknowledgment

I thank D. G. Iezzoni of the Clinical Research Department, Chas. Pfizer Co., Inc., Brooklyn, N. Y., for his interest and aid in the project reported in this article.

ORAL DIABETIC THERAPY - DIABINESE Conversion and Progress Report

Patient Name
C. N.
Chart No.
1637

1637

Date		Urine Sugar Test				Urine Keto-Test				NPH Insulin				Dosage Per Day	
		A.M.	A.M.	P.M.	P.M.	A.M.	A.M.	P.M.	P.M.	A.M.	A.M.	P.M.	P.M.		
1958															
Apr. 22			0		0		BS*	150					20		500 mg.
23			0		0								18		500
24			3		0								22		500
25			0		0								18		500
26			0		0								16		500
27			1		1								18		500
28			1		0								20		500
29			0		0								18		500
30			0		0								16		500
May 1			0		0								14		500
2			0		0								12		500
3			0		1								10		500
4			0		1								8		500
5			3		1								12		500
6			0		0								12		500
7			0		0								10		500
8			0		3								8		500
9			3		0								10		500
10			0		0								8		500
11			0		0								6		500
12			0		3								4		500
13			0		0								4		500
14			1		0		BS	138					6		500
Insulin discontinued on May 20, 1958															

* All blood sugars were performed at random by the Folin-Malmros capillary blood sugar method, and expressed in milligrams per 100 cc. of blood.

C. N., female, age 48, weight 180. A diabetic on insulin for 22 years and currently on 35 U. of NPH insulin and 1.0 gm. of Orinase. She was started on 250 mg. Diabinese and 20 U. NPH insulin daily and promptly became sugar-free. In 1 week she had an insulin reaction on one-half the previous insulin dose and gradually discontinued insulin.

ORAL DIABETIC THERAPY - DIABINESE

Conversion and Progress Report

[illegible]

* All blood sugars were performed at random by the Folin-Malmros capillary blood sugar method, and expressed in milligrams per 100 cc. of blood.

W. W., male, age 68, weight 174. A diabetic for 14 years, patient was taking 20 to 30 U. of NPH insulin. He was put on 20 units of NPH with 200 mg. of Diabinese daily. He continued sugar-free, gradually reducing his insulin and discontinuing it in less than 2 weeks.

ORAL DIABETIC THERAPY - DIABINESE																			
Conversion and Progress Report																			
Date		Urine Sugar Test						Urine Keto-Test						Insulin			Dosage Per Day		
		A.M.	A.M.	P.M.	P.M.	P.M.	P.M.	A.M.	A.M.	P.M.	P.M.	P.M.	A.M.	A.M.	P.M.				
1958							0					tr.						400 mg.	
May	22		1				1					tr.						400	
	23		1				1					0						400	
	24		1				1					0						400	
	25		1				1					0						400	
	26		1				0					0						200	
	27		0				0					0						200	
	28		0				0					0		BS	138			200	
	29		0				0					0						200	
	30		0				1					0						400	
	31		0				0					0						400	
June	1		1				0					0						400	
	2		0				0					0						200	
	3		0				0					0						200	
	4		1				0					0						400	
	5		0				0					0		BS	202			400	
	6		0				0											200	
	7		0				0											200	
	8		0				0											0	
	9		0				0												
	10		0				0												
	11		0				0							BS	189				
Diabinese discontinued 6/8/58																			

* All blood sugars were performed at random by the Folin-Malmros capillary blood sugar method, and expressed in milligrams per 100 cc. of blood.

H. M., female, age 47, weight 185. A diabetic for 2 years, and on diet without insulin; blood sugar 244 with 4 plus sugar. She was put on Diabinese, 200 to 400 mg. daily, and tests became sugar-free in a few days. At the end of 1 week her blood sugar was 138; Diabinese was discontinued in 2½ weeks.

EXPERIENCES WITH TOLBUTAMIDE AND CHLORPROPAMIDE IN TUBERCULOUS DIABETIC PATIENTS

Francis O. Segarra, David S. Sherman, and Benson S. Charif

Boston Sanatorium, Mattapan, Mass.

Although the literature regarding the action, use, and results of treatment of diabetes with tolbutamide (Orinase) is voluminous and detailed, there has been very little recorded about the use of this drug in the treatment of diabetes coexistent with active pulmonary tuberculosis. Inasmuch as these two diseases usually complicate each other, it seemed appropriate to study the effects of this oral antidiabetic drug in such cases. When the discovery of chlorpropamide 1-propyl-3-(*p*-chlorobenzenesulfonyl)urea was announced, a comparative study of the two drugs seemed to be a logical and desirable project.

Tuberculous Diabetic Patients: Treatment with Tolbutamide, House Diet, and Antituberculosis Chemotherapy

A group of 9 patients with far advanced active pulmonary tuberculosis and coexistent diabetes mellitus were treated with tolbutamide and standard house diet (about 3000 calories per day). Although all patients in this group had active tuberculosis, none had any signs of toxicity such as fever, chills, emaciation, or hemorrhage. Tuberculosis treatment in all cases consisted of daily isoniazid (INH) and *p*-aminosalicylic acid (PAS).

Seven patients were males; two were females; their ages ranged from 42 to 83 years.

Diabetic histories. In three patients the diabetes had been present for several years. One of these had been well controlled with 40 to 50 U. of NPH insulin (case 1). The other two cases (2 and 4) had been controlled by diet without insulin.

In four patients, the diabetes had been discovered recently; that is, simultaneously with the diagnosis of tuberculosis. Three of these had been controlled with NPH insulin in doses of 35 to 55 U. daily (cases 6, 8, 9), the fourth (case 7) required 75 U.

In two patients the diabetes was discovered during hospitalization for tuberculosis. One was found 21 days after admission (case 3) and the other, five months after admission (case 5).

All 9 patients had mild or moderate diabetes, with only one requiring 75 U. of insulin daily. None of these patients had ever had an episode of acidosis or coma. Although some of these patients were not ideal candidates for tolbutamide therapy because of their insulin requirements, they were included in the study to determine if any benefit might be derived from oral antidiabetic therapy.

Laboratory studies were made before the start of therapy, after one week of therapy, and after one month of therapy. These included total protein, A/G ratio, alkaline phosphatase, Bromsulphalein (BSP), white blood count

(WBC), and fasting blood sugar (FBS). The sugar was checked by 4 urine sugar tests daily and by frequent FBS studies.

The duration of tolbutamide therapy in all cases was too brief (22 days to 4 months) to determine any secondary failures. Insulin was omitted the day before starting the drug; then a daily dose of 2 gm. was given for 3 days, followed by daily doses of 1 gm. In cases that were not satisfactorily controlled with 1 gm. daily, the dose was gradually increased to 4 gm. daily before considering such a case a failure.

TABLE 1 represents a tabulated summary of this study. The penultimate column indicates that only one patient (case 3) was satisfactorily controlled by tolbutamide. In this very small series, this represents a 12 per

TABLE 1
TUBERCULOUS DIABETICS: "FREE DIET" AND TOLBUTAMIDE

	Patient	Sex	Age	Activity of tuberculosis	Diabetic history	Type of diabetes	Insulin units	Insulin control	Tolbutamide control	Duration on tolbutamide
1	B. M.	♂	63	Inact.	4 yr.	Mod.	40-50	Good	Poor	22 days
2	B. F.	♂	75	Mod.	9 yr.	Mod.	35-50	Good	Poor	3 mo.
3	H. C.	♀	59	Act.	1 mo.	Mild	30	Good	Good	4 mo.
4	J. B.	♂	83	Mod.	1 yr.	Mild	20-40	Good	Poor	3 mo.
5	E. M.	♀	54	Act.	2 mo.	Mod.	30-45	Good	Poor	3 mo.
6	K. X.	♂	76	Mod.	20 mo.	Mod.	40-50	Fair	Poor	1½ mo.
7	J. R.	♂	57	Mod.	9 mo.	Mod.	75	Good	Poor	1½ mo.
8	J. S.	♂	42	Act.	8 mo.	Mod.	35	Good	Poor	2 mo.
9	G. R.	♂	70	Act.	2 yr.	Mod.	55	Fair	Poor	2 mo.

cent rate of success. Therefore, we conclude that diabetic patients who require a "free" high-calorie diet for the treatment of their tuberculosis, even if the diabetes is only of moderate degree, are not good prospects for successful tolbutamide control. In such cases, insulin remains the drug of choice.

In all the cases studied, laboratory studies remained normal (with the exception of urine sugar and FBS in the failures), and no toxic side effects were observed.

Tuberculous Diabetic Patients on Restricted 2000-Calorie Diet: Treatment with Tolbutamide and Antituberculosis Drugs

Ten tuberculous diabetic patients were given a 2000-calorie diet containing: fat, 80 gm.; protein, 100 gm.; and carbohydrate, 200 gm. (TABLE 2). Five of the patients in this group were also members of the first group (unrestricted diet). The same preliminary studies were made and the same tolbutamide dosage was given.

Before starting tolbutamide, the single-dose test was made in all ten patients.

Except for case 7, who showed no response 4 hours following the ingestion of 3 gm. of Orinase, the results of the single-dose test varied from 18 to 52 per cent reduction of blood sugar, with an average of 28 per cent.

There was no correlation between the effect of the single-dose test and the

insulin requirements. However, there was some correlation between the result of the single-dose test and the adequacy of control of the diabetes by insulin. Thus, in the 3 cases (5, 7, and 8) in which insulin control was less than satisfactory, the results with the single-dose test were the poorest of the entire group, showing blood sugar decreases of 18, 0, and 21 per cent. The control of diabetes with tolbutamide was good in 4 patients and poor in 6.

If case 7 is excluded because of the severity and long-standing history of the diabetes, only fairly controlled by insulin and showing no response at all to the single-dose test, we have 4 of the 9 patients well controlled with 1 gm. of tolbutamide daily. In other words, in tuberculous diabetic patients on a

TABLE 2
TUBERCULOUS DIABETICS: 2000-CALORIE DIET AND TOLBUTAMIDE

	Patient	Age	Sex	Activity of tuberculosis	Diabetic history	Type of diabetes	Insulin units	Insulin control	Tolbutamide single-dose test,* %	Tolbutamide control	Duration on tolbutamide
1	B. F.	75	♂	Mod.	9 yr.	Mod.	30-45	Good	52	Poor	12 days
2	H. C.	60	♀	Act.	9 mo.	Mild	30	Good	26	Good	1 mo.
3	J. B.	83	♂	Mod.	16 mo.	Mod.	20-40	Good	24	Poor	2 mo.
4	E. M.	55	♀	Act.	8 mo.	Mild	30	Good	43	Good	1 mo.
5	K. X.	76	♀	Mod.	2 yr.	Mod.	30-50	Fair	18	Poor	1 mo.
6	W. R.	73	♂	Act.	6 yr.	Mild	25	Good	41	Good	1 mo.
7	C. A.	52	♀	Act.	27 yr.	Severe	30-60	Fair	0	Poor	10 days
8	A. C.	61	♀	Act.	6 yr.	Mod.	40-45	Fair	21	Poor	1 mo.
9	J. D.	66	♂	Act.	10 mo.	Mild	25	Good	40	Good	1 mo.
10	A. B.	47	♂	Act.	2½ yr.	Mod.	55-70	Good	40	Poor	1 mo.

* Reduction of blood sugar.

restricted and regulated 2000-calorie diet, tolbutamide was effective in 45 per cent of the cases, as opposed to 12 per cent effectiveness in the group on an unrestricted diet.

It is of interest to note that those patients whose insulin requirements were 30 U. or less were well controlled with tolbutamide (cases 2, 4, 6, and 9). On the other hand, those requiring more than 30 U. of insulin for good control of their diabetes did not respond well to the oral drug (cases 1, 3, 5, 7, 8, 10). This "ceiling" of 30 U. seems to be definite and fixed.

In this group, too, no toxic side effects were observed.

Tuberculous Diabetic Patients Treated with Chlorpropamide, 2000-Calorie Diet, and Antituberculosis Drugs

Chlorpropamide was used as an oral antidiabetic agent in patients with coexistent active pulmonary tuberculosis and diabetes. This group was almost identical in all respects to the group of the preceding section; seven of this group were also in the tolbutamide 2000 calorie diet group of the same section (cases 1, 2, 3, 4, 5, 6, 7).

The dosage schedule of chlorpropamide was as follows: initially, 500 mg. daily for 3 days; increments in amounts of 200 mg. were made at 3-day inter-

vals if the given dose was inadequate to control the diabetes. The maximum dose was 1000 mg. daily. The drug was given in a single dose. If the maximum dose (1000 mg.) failed to provide satisfactory control after 3 days, it was discontinued and the case was termed a failure.

The results of this study are tabulated in TABLE 3. Six patients showed a good response; 4 patients were not well controlled. If case 1 of this group is omitted from consideration (this is case 7 of the tolbutamide group) because of the severe degree and longstanding history of diabetes, chlorpropamide was successful in the control of 6 of 9 patients. It is interesting to note that the six patients who were well controlled with chlorpropamide were patients

TABLE 3
TUBERCULOUS DIABETICS: 2000-CALORIE DIET AND CHLORPROPAMIDE

	Patient	Sex	Age	Activity of tuberculosis	Diabetic history	Type of diabetes	Insulin units	Insulin control	Chlorpropamide control	Duration on chlorpropamide
1	C. A.	♀	52	Act.	27 yr.	Severe	30-60	Fair	Poor	12 days
2	H. C.	♀	60	Act.	10 mo.	Mild	30	Good	Good	35 days
3	E. M.	♀	55	Act.	9 mo.	Mild	30	Good	Good	75 days
4	A. C.	♀	61	Act.	6 yr.	Mod.	40-45	Fair	Poor	75 days
5	K. X.	♂	77	Mod.	2 yr.	Mod.	30-50	Fair	Poor	14 days
6	A. B.	♂	47	Act.	2½ yr.	Mod.	55-70	Good	Poor	7 days
7	W. R.	♂	73	Act.	6 yr.	Mild	25	Good	Good	30 days
8	K. T.	♀	55	Act.	2 mo.	Mild	25	Good	Good	7 days
9	W. C.	♂	63	Mod.	2 mo.	Mild	25	Good	Good	45 days
10	M. S.	♀	64	Act.	1 yr.	Mild	30	Good	Good	45 days

whose disease had previously been well controlled with no more than 30 U. of insulin. Patients whose insulin requirements were greater than 30 U. did not respond adequately.

No untoward laboratory changes occurred during therapy. There was one instance (case 7) of dizziness, nervousness, and irritability. However, it must be pointed out that this patient is an elderly man, with considerable senile degenerative changes in addition to the two chief presenting diseases.

Comparative Results of Tolbutamide and Chlorpropamide Therapy

In evaluating the results of treatment with the two oral antidiabetic agents, it was felt that the record of one patient whose medical background is so severe, varied, and complex should be eliminated from consideration. Since she was in both the tolbutamide and chlorpropamide groups, the elimination of this patient from the comparative evaluation does not unbalance either group.

At first sight, the difference between the 45 per cent success of the tolbutamide therapy group and the 67 per cent success of the chlorpropamide group seems very striking. However, further analysis reveals that the criterion for success with either drug seems to be the degree of diabetic deficiency and the consequent insulin requirements for control.

In the tolbutamide group, 4 of the 9 patients were well controlled by 30 U. of insulin or less. These patients responded well to tolbutamide. The

remaining six patients, who required more than 30 U. of insulin, did not respond well.

In the chlorpropamide group, 6 of the 9 patients had disease that was controlled with 30 U. or less of insulin. These six patients showed a good response to chlorpropamide, and were well controlled. The remaining three cases had previously required more than 30 U. for adequate control, and these patients did not do well on chlorpropamide.

From these observations, we conclude that in our small groups of tuberculous diabetic patients the efficacy of both drugs is dependent on the mild or moderate nature of the disease. Those patients requiring less than 30 U. of insulin for control do well with either drug. This conclusion is further supported by the fact that, of the seven patients who were studied in both drug groups, the same patients did well with both drugs and the remaining patients did badly with both drugs.

Although our studies were limited to a small number of patients, we feel that we can state as a clinical guide that a tuberculous diabetic patient, even with far advanced pulmonary disease, who is not toxic and who is on a 2000-calorie diet will do well with either tolbutamide or chlorpropamide if his insulin requirement for control is 30 U. or less. On the other hand, patients requiring even a little more than 30 U. failed to respond to either drug.

The Action of Isoniazid on Carbohydrate Metabolism

Isoniazid (isonicotinic acid hydrazide) has been for the past several years one of the drugs commonly used in the treatment of tuberculosis. There has been only rare mention of the effect of INH on carbohydrate metabolism and on the fasting blood sugar; these reports have been primarily in the foreign literature. We believe that our preliminary observations (TABLE 4) in this area might be of more than academic interest and that this subject merits further investigation.

TABLE 4
HYPOGLYCEMIA EFFECT OF ISONIAZID

	Patient	Sex	FBS mg. per cent	BS 4 hr. after 500 mg. INH		FBS mg. per cent	BS 4 hr. after tolbutamide 3 gm.		FBS mg. per cent	BS 4 hr. after tolbutamide and INH	
				mg. per cent	% de- crease		mg. per cent	% de- crease		mg. per cent	% de- crease
1	J. B.	♂	327	285	13	384	293	24	259	171	34
2	A. B.	♂	191	182	5	217	130	40	222	126	43
3	K. X.	♂	327	285	13	318	259	19	370	309	17
4	C. A.	♀	450	443	0	490	490	0	467	467	0
5	H. C.	♀	201	146	26	176	130	26	163	101	38
6	E. M.	♀	234	154	34	271	154	43	278	118	57
7	A. C.	♀	420	359	15	443	348	21	348	285	18
8	J. D.	♂	182	201	10*	212	126	41	150	104	30

Average reduction of blood sugar after 500 mg. INH = 18 per cent.

Average reduction of blood sugar after 3 gm. tolbutamide = 28 per cent.

Average reduction of blood sugar after INH and tolbutamide = 35 per cent.

* 10 per cent increase rather than a decrease.

In a group of 8 tuberculous diabetic patients, the following procedures were performed: (1) tolbutamide single-dose test using 3 gm. of the drug; (2) a similar test using 500 mg. of INH (with determination of the FBS before, and 4 hours after, ingestion of 500 mg. of INH); and (3) a similar test using both 3 gm. of tolbutamide and 500 mg. INH given simultaneously.

Results. The tolbutamide single-dose tests produced blood sugar reductions ranging from 19 to 43 per cent, with an average decrease of 28 per cent of the initial FBS level. One patient showed no response.

In the INH single-dose test one patient showed an increase in the FBS of 10 per cent. The patient who showed no response to tolbutamide showed no response to INH. The remaining 6 patients showed a significant reduction of the blood sugar, ranging from 5 to 34 per cent, with an average reduction of 18 per cent.

When the combined single-dose test (3 gm. Orinase and 500 mg. of INH) was given to the same group, there were reductions in the blood sugar ranging from 17 to 57 per cent, with an average of 35 per cent.

From these studies it is apparent that INH has a definite effect on carbohydrate metabolism; in almost every case INH exerts a hypoglycemic effect that is not an insignificant one, and when combined with tolbutamide, seems to enhance the action of this drug.

These observations suggest an area of investigation that hitherto has not been reported in the search for oral control of diabetes.

Summary

The efficacy of two oral antidiabetic agents in cases of diabetes with coexistent pulmonary tuberculosis (active, but not toxic) was studied and compared. These two agents are tolbutamide and chlorpropamide. The salient observations resulting from this study are as follows.

The results of treatment with tolbutamide and chlorpropamide in tuberculous diabetics are essentially the same.

Tuberculous diabetic patients who require free diet ("house diet"), because of their tuberculosis are not good candidates for treatment with either of the oral antidiabetic agents. This is true even if the diabetes is mild.

Tuberculous diabetic patients on a 2000-calorie diet show good response to both tolbutamide and chlorpropamide in cases of mild diabetes that were previously controlled by 30 U. of insulin or less. It is interesting to note that, if insulin requirements were even slightly more than 30 U., neither drug was effective in maintaining adequate control.

The extent of tuberculosis did not seem to influence the activity of either drug. All patients studied have active disease, but were not toxic.

Preliminary studies of the effects of isoniazid, the most commonly used drug today in the chemotherapy of tuberculosis, on the blood sugar, revealed a significant hypoglycemic effect in almost every case. When compared with one of the oral antidiabetic drugs (tolbutamide), the hypoglycemic effect was greater than with either INH or the antidiabetic drug alone. In other words, isoniazid enhanced the hypoglycemic effect of the antidiabetic drug. This suggests further possibilities for the oral drug control of diabetes.

THE ROLE OF A TOLBUTAMIDE TOLERANCE TEST IN THE DETECTION OF THE MILD DIABETIC STATE: A PRELIMINARY REPORT

Harold Zarowitz and Benjamin Eis

Diabetes Division, Jewish Hospital of Brooklyn, Brooklyn, N. Y.

The detection of mild diabetes mellitus, which is asymptomatic in nature, usually requires the oral glucose-tolerance test. This procedure, at its best, has not been completely satisfactory because of such extraneous factors as diet, exercise, and gastrointestinal absorption, which modify the results. Another parameter for detecting mild diabetes may be necessary, and it seems that the tolbutamide-tolerance test may be that parameter. Experimental evidence indicates that other sulfonylurea compounds will give the same response.

In attempting to assess the response to oral tolbutamide therapy, Braverman¹ injected tolbutamide intravenously in a few patients and demonstrated a distinct difference in response between nondiabetics and mild diabetics. This response consisted in a rapid drop of the blood sugar in the nondiabetic group and a lesser, although sustained drop, in the diabetic group. In a larger series Unger and Madison² recently demonstrated a similar response.

Because we have had the problem of interpreting equivocal glucose-tolerance tests in a large hospital, we became interested in evaluating the tolbutamide response as a means of separating the nondiabetic from the mild diabetic group. This problem is of particular importance in pregnancy.

Subjects and Methods

Our initial study was performed on a total of 69 patients: 25 nondiabetics, 26 proved mild diabetics, 9 pregnant nondiabetics, and 9 pregnant diabetics. Following Moyer's³ criteria, diabetes mellitus was diagnosed if the 2-hour blood sugar level, determined by the Folin-Wu method,⁴ was more than 140 mg. per cent. There are no patients with active liver or thyroid disease in our entire group. None of the diabetic patients were receiving or required insulin. Three-hour glucose-tolerance tests were performed initially on all patients. After having been on a free diet for a few days, they reported to our clinic for the tolbutamide test. One gram of sodium tolbutamide was diluted in 20 cc. of isotonic saline solution and then injected intravenously within 2 min. after the withdrawal of a fasting blood specimen. Subsequently a blood specimen was withdrawn every 20 min. for as long as 2 hours and analyzed for blood sugar, in duplicate by the Folin-Wu method. Because we realized that glucose tolerance can change considerably through the course of pregnancy, the tolbutamide tests were done in the 2 pregnant groups not later than 10 days after the glucose-tolerance test. Some were done both in the intra- and postpartum periods.

Results

In the groups of 25 nondiabetic patients (FIGURE 1, upper graph), the greatest fall in blood sugar occurred 40 min. after the injection of sodium tolbutamide. The mean percentage fall, expressed as percentage fall of the fasting blood sugar, was 40 per cent, with a range of 17 to 62 per cent. At 20 min., the mean fall was 36 per cent with a range of 15 to 72 per cent. At

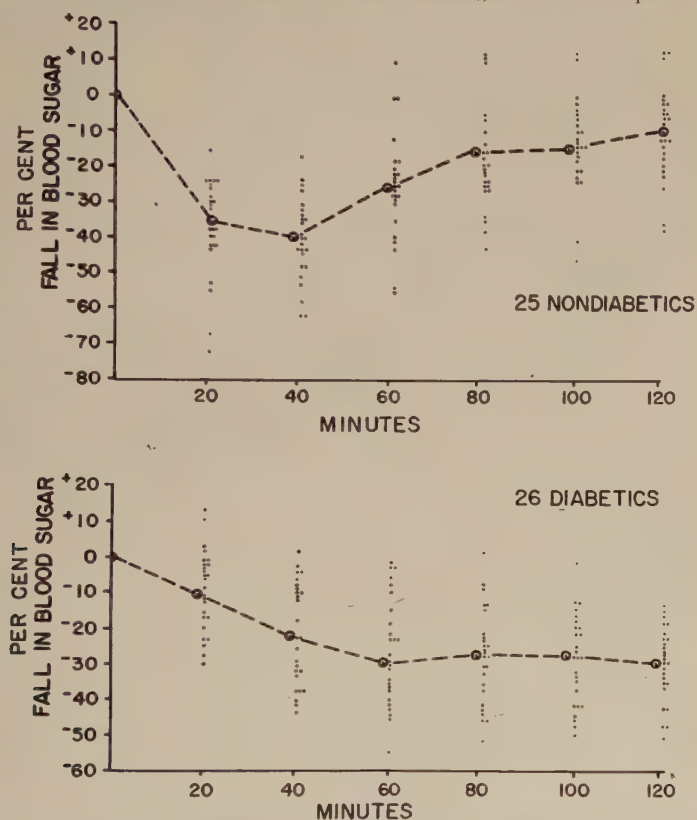


FIGURE 1. The mean tolbutamide tolerance curves of twenty-five nondiabetics and twenty-six diabetics. Individual responses are recorded.

60 min., the mean fall was 26 per cent with a range from a 10 per cent rise to a 55 per cent fall. At the 80-, 100-, and 120-min. intervals, the mean percentage fall decreased to 16, 16, and 10 per cent, respectively. Of these 25 patients, 7 (28 per cent) demonstrated the maximal fall at 20 min., 17 (68 per cent) at 40 min., and 1 (4 per cent) at 60 min. Thus, it is possible to state that the normal response to intravenous sodium tolbutamide is characterized by a prompt fall in the blood sugar, most frequently greatest within 40 min., and by a good degree of hypoglycemic responsiveness after 60 min. In the group of 26 diabetic patients (FIGURE 1, lower graph) the

greatest fall in blood sugar levels occurred at the 60-min. interval and continued to 120 min. The mean percentage fall at 60 min. was 30 per cent, with a range of 3 to 55 per cent. At 20 min., the mean percentage fall was 11 per cent, with a range from a 10 per cent rise to a 30 per cent fall. At 40 min., the mean percentage fall was 23 per cent with a range from a 1 per cent rise to a 44 per cent fall. It is to be noted that, at this time interval,

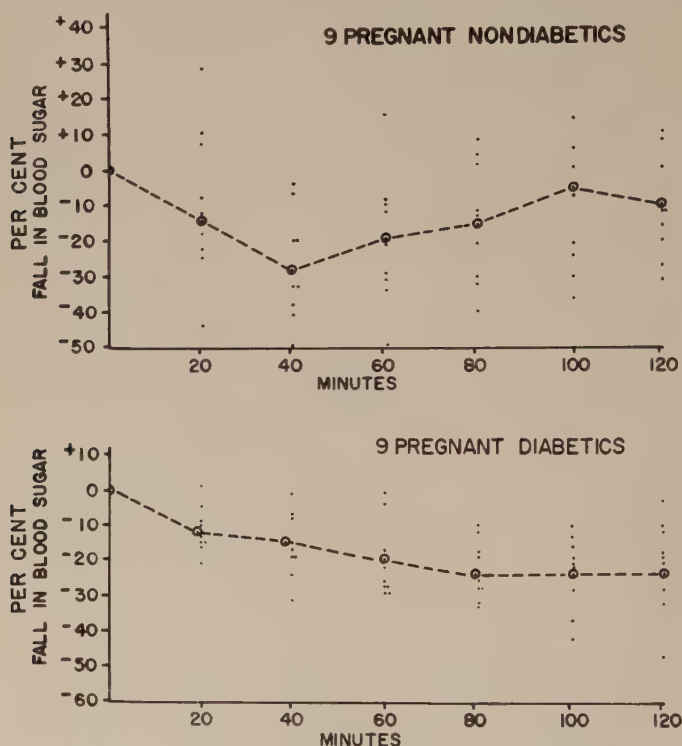


FIGURE 2. The mean tolbutamide tolerance curves of nine pregnant nondiabetics and nine pregnant diabetics. Individual responses are recorded.

the blood sugar levels of 3 patients (11 per cent of this group) were able to fall below the greatest mean percentage fall of the nondiabetic group. Thus, there is some degree of overlapping. However, in these 3 patients the blood sugar levels continued to fall until the end of the test. Thus, the response of the diabetic group is characterized by a more gradual fall, reaching its maximum at 60 min. or more, and demonstrating no significant hypoglycemic responsiveness.

The response to intravenous tolbutamide is not altered in pregnancy (FIGURE 2, upper graph). In a group of 9 nondiabetic pregnant patients, the greatest mean percentage fall was 28 per cent, and occurred at 40 min. The range of variation was 4 to 52 per cent. They also demonstrated a considerable degree of hypoglycemic responsiveness. In the group of 9 preg-

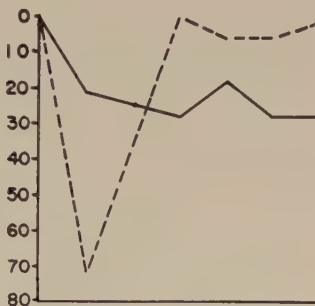
nant diabetic patients, the greatest mean percentage fall occurred at 80 min., with a range of 10 to 35 per cent. Within 120 min., there was no hypoglycemic responsiveness of any significance. Of these 9 patients, only 1 whose glucose tolerance was abnormal demonstrated a normal tolbutamide response.

CASE I**GLUCOSE TOLERANCE TESTS:**

	7 MONTHS	POSTPARTUM
F	86	113
1/2 HR.	160	193
1 HR.	172	133
2 HR.	142	112
3 HR.	130	102

TOLBUTAMIDE CURVE

———— INTRAPARTUM
 - - - - - POSTPARTUM

**CASE II****GLUCOSE TOLERANCE TESTS:**

	7 MONTHS	POSTPARTUM
F	81	77
1/2 HR.	131	139
1 HR.	144	151
2 HR.	146	86
3 HR.	53	74

TOLBUTAMIDE CURVE

———— INTRAPARTUM
 - - - - - POSTPARTUM

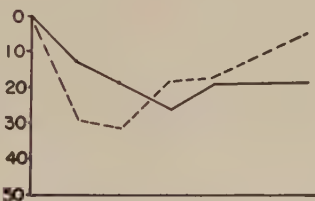


FIGURE 3. Curves of two patients whose mildly abnormal glucose and tolbutamide tolerance at seven months' pregnancy was reversed completely in the postpartum period.

In 2 instances, both glucose tolerance and tolbutamide curves were studied in the intra- and postpartum periods (FIGURE 3). The upper part of the chart demonstrates a mildly abnormal glucose-tolerance curve characterized by a blood sugar of 142 mg. per cent at 2 hours. The tolbutamide curve is also considered to be abnormal, demonstrating both a delayed fall and no significant return. The postpartum glucose-tolerance test shows a reversion toward normal. The tolbutamide curve shows a completely normal response characterized by a rapid fall and a rapid return. The lower section of the chart shows a similar situation where both abnormal glucose tolerance and tolbutamide curves at 7 months reverted to normal in the postpartum period.

Discussion

It is only in instances of suspicious mild diabetes, either in or out of pregnancy, that it is difficult to arrive at a definite diagnosis. Under these cir-

cumstances, additional measures beyond the glucose-tolerance test have been wanting. The response of the blood sugar in a 2-hour period to an intravenous injection of sodium tolbutamide separates the mild diabetic from the normal patient if 2 conditions are fulfilled: first, the fall in blood sugar should be prompt and should reach its lowest level by 40 min. and, second, there should be a significant degree of hypoglycemic responsiveness within the remaining 80 min. These conditions have been suggested because it has been demonstrated that, although some diabetic patients are able to respond promptly with a brisk fall in blood sugar levels, the remainders of their curves are also in a downward direction. In a very few instances was there a minimal irregular upward turn. Analysis of the latter part of many more curves, it is hoped, will allow the degree of hypoglycemic responsiveness to be expressed mathematically. We should like to emphasize the fact that Moyer's glucose-tolerance criteria clearly separated the normal from the abnormal when correlating the tolerance curves with the tolbutamide curves. There were no patients with blood sugars higher than 140 mg. per cent at 2 hours who had a normal tolbutamide response. Nine patients with distinctly abnormal tolbutamide curves had 2-hour blood sugars between 140 mg. per cent and 160 mg. per cent.

The procedure is not associated with any significant side effects. Only two patients had, early in the test, hypoglycemic symptoms that required the administration of glucose.

We feel that further experience with this procedure will permit greater accuracy in detecting the mild diabetic state.

Summary

- (1) Intravenous tolbutamide tolerance tests were performed on 69 patients.
- (2) The normal response in a 2-hour period is characterized by a prompt fall in the blood sugar followed by a significant hypoglycemic responsiveness. The mild diabetic response demonstrates a lesser fall with no significant hypoglycemic responsiveness.
- (3) Pregnancy does not alter the tolbutamide response in normal patients.
- (4) It is felt that this procedure will help in detecting the mild diabetic state.

Acknowledgment

We express our thanks to Martin G. Goldner of the Jewish Chronic Disease Hospital, Brooklyn, N. Y., for his suggestions.

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THE INTRAVENOUS TOLBUTAMIDE RESPONSE TEST IN THE DIAGNOSIS OF MILD DIABETES

Roger H. Unger and Leonard L. Madison

Department of Medicine, The University of Texas Southwestern Medical School,
and Veterans Administration Hospital, Dallas, Texas.

The striking difference in response to intravenously administered sodium tolbutamide exhibited by nondiabetics and mild diabetics has been reported in detail elsewhere.^{1,2} More recently the clinical usefulness of a standardized

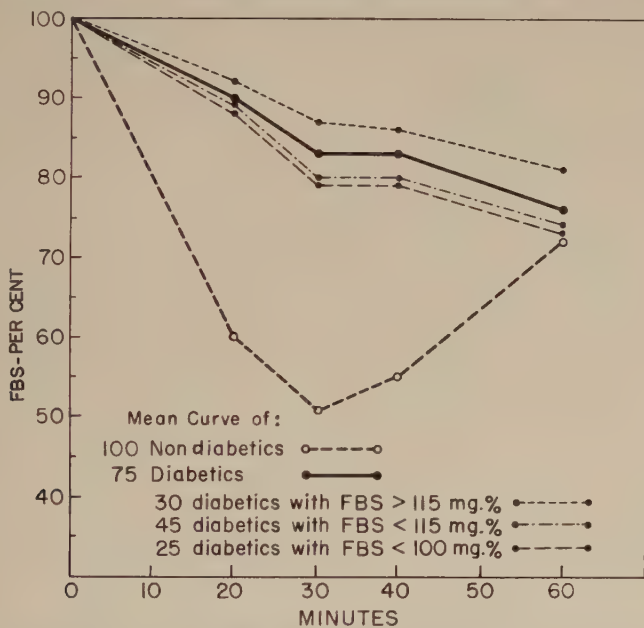


FIGURE 1. Mean tolbutamide response curves of all but five of the subjects studied, illustrating the blood glucose responses typical of nondiabetics and of mild diabetics of varying degrees of severity. The curve of 45 diabetics with fasting blood sugar (FBS) <115 mg. per cent includes the 25 with FBS <100 mg. per cent, whose mean curve is shown separately. Five of the 79 diabetics in this study are not represented in this figure, but their omission does not perceptibly affect the results. Reprinted with the permission of the *Journal of Clinical Investigation*, Volume 37, 1958.

intravenous tolbutamide response test in the diagnosis of mild diabetes mellitus has been delineated and diagnostic standards suggested.³ This paper, therefore, will merely review briefly certain aspects of these earlier reports as they pertain to the experiences of Zarowitz and Eis⁴ with this test.

Zarowitz and Eis have provided further valuable evidence to support the qualifications of the intravenous tolbutamide response test as a diagnostic procedure for mild diabetes. In addition, they have extended experience with the test to the pregnant state. The correlation between reversible

abnormal tolbutamide response curves and reversible abnormal glucose tolerance curves in the intra- and postpartum periods, which they have observed, is consonant with the hypothesis that both tests measure the same physiological function.

Clinical usefulness of a diagnostic test for mild diabetes mellitus depends on its ability to differentiate the diabetic without fasting hyperglycemia from the nondiabetic. That the intravenous tolbutamide test qualifies in this respect is indicated by the data in FIGURE 1. It will be noted that the response curve of the mildest diabetics with fasting normoglycemia closely

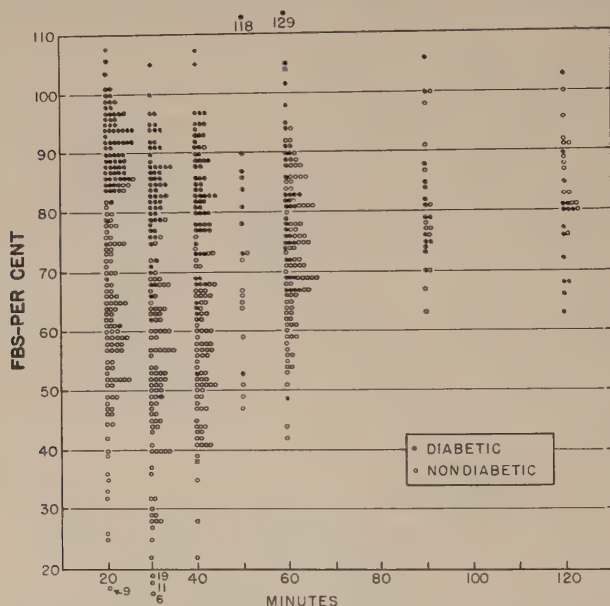


FIGURE 2. Data on the tolbutamide response tests of 100 nondiabetic and 79 mildly diabetic subjects. Blood glucose levels are expressed as percentages of the fasting blood sugar (FBS) concentration. Separation of the two groups is maximal at 20 and 30 min. Reprinted with the permission of the *Journal of Clinical Investigation*, Volume 37, 1958.

parallels the gently sloping curve of the more severe diabetics and is in distinct contrast to the rapid fall in blood glucose level typical of the nondiabetic group. This fall reaches a nadir between 20 and 40 min. and is generally followed by a vigorous rebound to or toward the pretest value. Zarowitz and Eis report similar results.

Zarowitz and Eis have emphasized the diagnostic value of the rebounding blood glucose concentration which, as they point out, seldom occurs except in nondiabetics. Therefore, they extend the test beyond the 40-min. point in order to evaluate this segment of the curve. While there is no doubt as to the extremely high frequency of such rebounds in the nondiabetic group, we have regarded this phenomenon as being secondary to the more rapid decline in blood glucose level exhibited by the group, that is, the result of counterregulatory forces thus set into play. Rebound, in our opinion,

reflects both the rate and extent of decline in blood glucose level and the hypoglycemia responsiveness of the individual, rather than beta-cell function. Inasmuch as the rationale of the test is based on the presumed ability of tolbutamide to stimulate the release of insulin from the beta cell (this being effected more rapidly or to a greater degree in the beta cells of nondiabetic individuals), the descending limb of the tolbutamide response curve would be expected to mirror most directly beta-cell responsiveness to this stimulus.

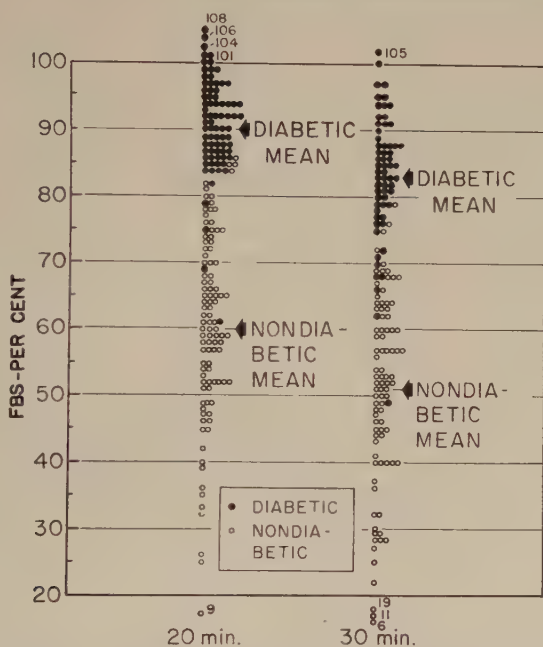


FIGURE 3. A close-up of FIGURE 2, showing the 20- and 30-min. specimens on the basis of the results at 20 min. It is felt that failure to decline to less than 84 per cent of the fasting blood sugar (FBS) is strong evidence for the diagnosis of diabetes mellitus, while the ability to respond with a decline in blood sugar to 80 per cent or more of the FBS makes the diagnosis of diabetes unlikely. Between 80 and 84 per cent is a presumptively abnormal but nondiagnostic zone. Reprinted with the permission of *Diabetes*, Volume 7, 1958.

In FIGURE 2, in which the tolbutamide response curves of 100 nondiabetics and 75 mild diabetics are recorded, maximum separation of the 2 groups occurs at 20 and 30 min. A close-up of these points is provided in FIGURE 3. At 20 min., 95 per cent of the diabetic group have a blood glucose level of 80 per cent or more of the pretest value, whereas 96 per cent of the nondiabetic group have a level of less than 84 per cent of that value. Selection of 84 per cent and more as the diabetic zone, and 80 per cent and less as the nondiabetic zone, has provided standards that have thus far been 95 per cent accurate. The zone between 80 and 84 per cent of the pretest level is considered presumptively abnormal but nondiagnostic.*

* These standards apply only if blood glucose is determined by the Somogyi-Nelson technique. It is hoped that the data of Zarowitz and Eis will provide diagnostic standards for the Folin-Wu method.

The 30-min. specimen (FIGURE 3) appears to be diagnostically more specific inasmuch as 99 per cent of the nondiabetics are at less than 77 per cent of the pretest level by this time. However, this specimen is far too insensitive to be of value in the exclusion of diabetes, since 15 per cent of mild diabetics have reached the nondiabetic zone at this point.

It would seem that the blood glucose response within the first 30 min. of the tolbutamide injection provides the necessary diagnostic information in the vast majority of individuals. Termination of the test at this point

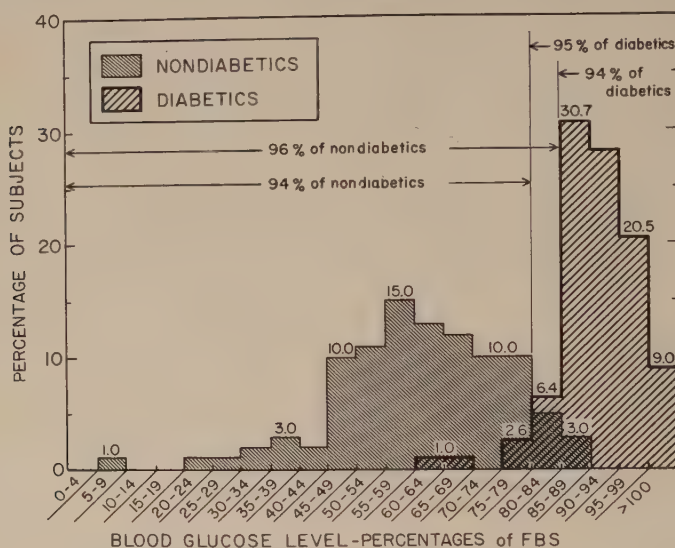


FIGURE 4. The frequency distribution of the blood glucose levels [in percentages of fasting blood sugar (FBS)] of all nondiabetics and diabetics in this study 20 min. after sodium tolbutamide injection. Overlap is limited essentially to the 75 to 89 per cent range and is maximal between 80 and 84 per cent. The selection of 85 per cent of fasting blood sugar or higher as the diabetic zone and less than 80 per cent as the nondiabetic provides a diagnostic accuracy of 94 per cent. The intervening zone of 80 to 84 per cent of FBS is considered to be presumptively abnormal but nondiagnostic. Reprinted with the permission of *Diabetes*, Volume 7, 1958.

endows it with an asset unique among diagnostic tests for mild diabetes, namely, brevity. Furthermore, it shortens the period of hypoglycemia that occurs in the nondiabetic group. If, however, the data of Zarowitz and Eis should indicate that examination of the rebound phase of the curve provides an unmistakable increase in diagnostic accuracy, prolongation of the test may be necessary.

As in the case of the glucose tolerance test^{5,6} it would be naive to expect that the tolbutamide response test can provide a sharp demarcation between diabetics and nondiabetics. Elements of these two groups will overlap to form a "gray" or nondiagnostic zone in which probability of disease can be roughly estimated, but in which accurate diagnosis is impossible. In FIGURE 4 the frequency distribution of 20-min. results is given for both groups. The

zone of overlap includes 10 per cent of each group and extends from 75 to 89 per cent of pretest level. Within this zone rigid adherence to the recommended diagnostic criteria can lead to error, since only probability of disease can be estimated. For example, these limited data suggest that among persons whose levels fall to between 75 and 79 per cent of the pretest value the diagnostic probability of diabetes is 21 per cent; between 80 and 84 per cent of pretest level it is 56 per cent; and between 85 and 89 per cent it is 91 per cent. Above and below the overlap zone diagnostic accuracy approaches 100 per cent.

It is our tentative impression that the zone of overlap of the tolbutamide response test is somewhat smaller than that of the oral glucose tolerance test, due primarily to fewer false positive results. It would be premature, however, to pass final judgment on the standards proposed for the tolbutamide response test in view of the controversy that still prevails in respect to the oral glucose tolerance test despite several decades of extensive use.

At present, however, the intravenous tolbutamide response test appears to qualify as an accurate, safe, rapid, and not unpleasant diagnostic procedure for mild diabetes that compares favorably with other methods now commonly used for this purpose.

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A CLINICAL COMPARISON OF CHLORPROPAMIDE AND TOLBUTAMIDE*

Peter H. Forsham, George J. Magid,† and David E. Dorosin‡

*Department of Medicine, the Metabolic Unit for Research in Arthritis and Allied Diseases,
University of California School of Medicine, San Francisco, Calif.*

Chlorpropamide, a recent orally administered hypoglycemic agent, has been compared to tolbutamide with regard to acute and chronic hypoglycemic potency and clinical effectiveness. The chemistry and animal studies of these two sulfonylureas are discussed elsewhere in this monograph.

Material

Studies were conducted on 26 subjects, including 1 normal and 25 diabetic. Five of the diabetics were hospitalized and maintained throughout their course on a constant diet of known composition with a daily intake of carbohydrate ranging from 150 to 220 gm. and protein from 80 to 120 gm. Both acute and chronic potency of the drugs were studied under these conditions. The normal subject was allowed a free diet between comparative studies of the 3.0-gm. dose. Sufficient time was allowed between the acute dosages of each drug to prevent interference with the subsequent hypoglycemic response. One of the diabetics had a previous total bilateral adrenalectomy and another had diabetes secondary to a total pancreatectomy. Sixteen of the diabetics were studied for 5 months as outpatients in the diabetes clinic of the University of California School of Medicine.

Methods

Blood sugars were measured by the method of Nelson and Somogyi.¹ Urinary glucose was measured by the glucose oxidase method.² Serum chlorpropamide levels were determined by a procedure developed by the Chemical Research and Development Department of Chas. Pfizer & Co., Inc. Brooklyn, N. Y.³ The content of unesterified fatty acids in the blood was obtained by the Davis extraction procedure,⁴ followed by titration according to the method of Dole.⁵

Results

Acute hypoglycemic effects. Various amounts of chlorpropamide and tolbutamide were administered as a single dose to both normal and diabetic subjects in order to compare the acute hypoglycemic effectiveness of each. Serum values of chlorpropamide and blood sugar levels were measured

* The study reported in this paper was aided by a grant from Chas. Pfizer & Co., Inc., Brooklyn, N. Y.

† Trainee of the National Institute of Arthritis and Metabolism; Research Fellow of the Metabolic Unit, Department of Medicine.

‡ Trainee of the National Institute of Arthritis and Metabolism; Research Fellow of the Metabolic Unit, Department of Medicine. Present address: New York Hospital, New York, N. Y.

simultaneously to study the acute relationship between the hypoglycemic effect and the concentration of the drug in the blood, and to observe the degree and rapidity of absorption. In FIGURE 1 the effect of the 0.5-gm. dose on 3 diabetic patients is noted. The blood sugars are measured in the fasting state for the first 4 hours. It is apparent that the onset of hypoglycemia is within the first 2 hours with both drugs and continues for 4 hours

0.5-GM. P.O. SINGLE DOSE

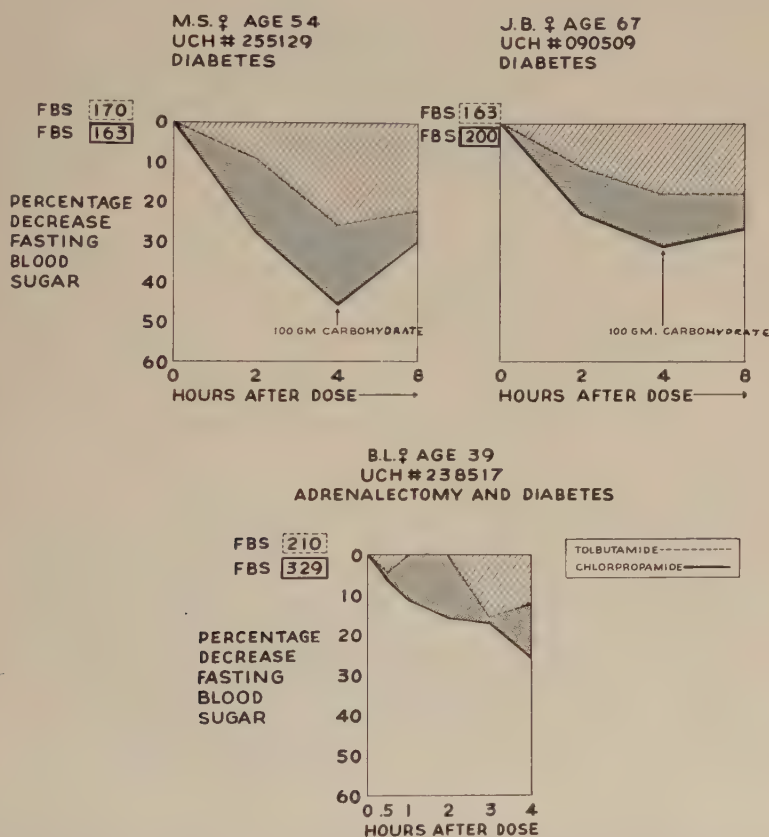


FIGURE 1. Comparative acute hypoglycemic potency of chlorpropamide and tolbutamide following 0.5 gm. as single oral dose.

before the standard meal is given. Postprandially there is a rise toward base line level. The comparative hypoglycemic potency of chlorpropamide is greater in each case than that of tolbutamide by a factor approaching 2 when the areas representing blood sugar fall are compared. In the lower part of FIGURE 1 the effect of a similar dose given orally to an adrenalectomized patient maintained on cortisone replacement alone shows a minimal response to tolbutamide, becoming significant 3 hours after the dose is given.

1.0-GM. P.O. SINGLE DOSE

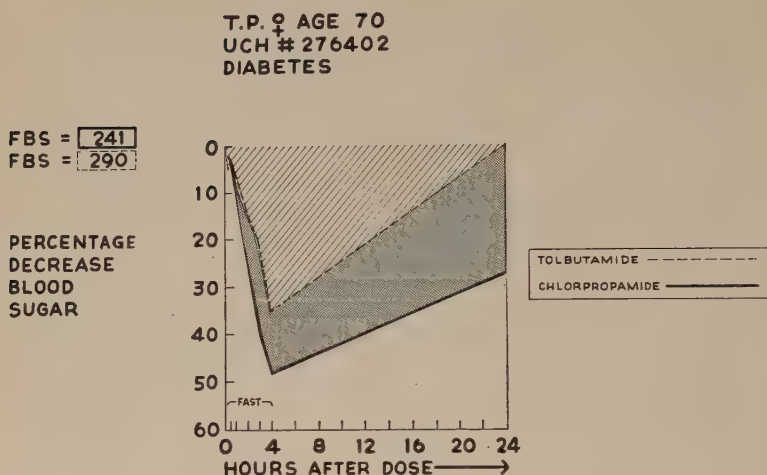


FIGURE 2. Comparative acute hypoglycemic potency of chlorpropamide and tolbutamide following 1.0 gm. as single oral dose.

3.0-GM. P.O. SINGLE DOSE

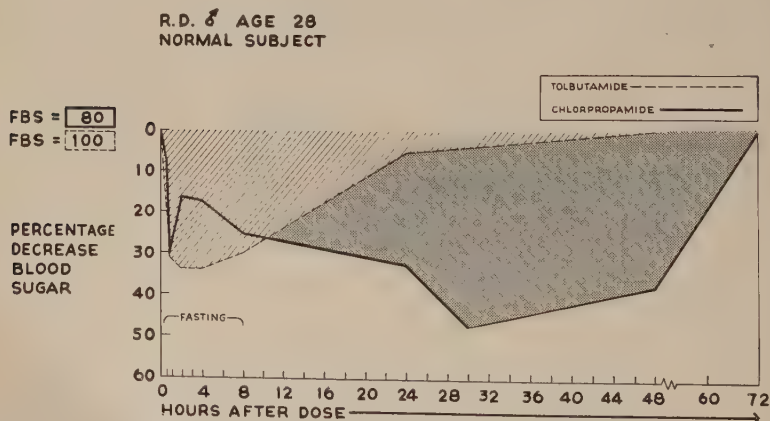


FIGURE 3. Comparative acute hypoglycemic potency of chlorpropamide and tolbutamide following 3.0 gm. as single oral dose.

Chlorpropamide, however, causes a much more significant fall starting within 30 min. after oral administration. Here the acute hypoglycemic potency of chlorpropamide is greater than that of tolbutamide by a factor greater than 2.

FIGURE 2 illustrates the comparative effect of the 1.0-gm. dose of the

2 compounds. Again it may be noted that chlorpropamide has a hypoglycemic potency approaching 2 times that of tolbutamide when the areas of hypoglycemia for similar time intervals are measured.

In FIGURE 3 the 3-gm. dose of each compound administered to a normal subject demonstrates that at this dose level the comparative hypoglycemic potency of chlorpropamide is greater than 2 times that of tolbutamide when

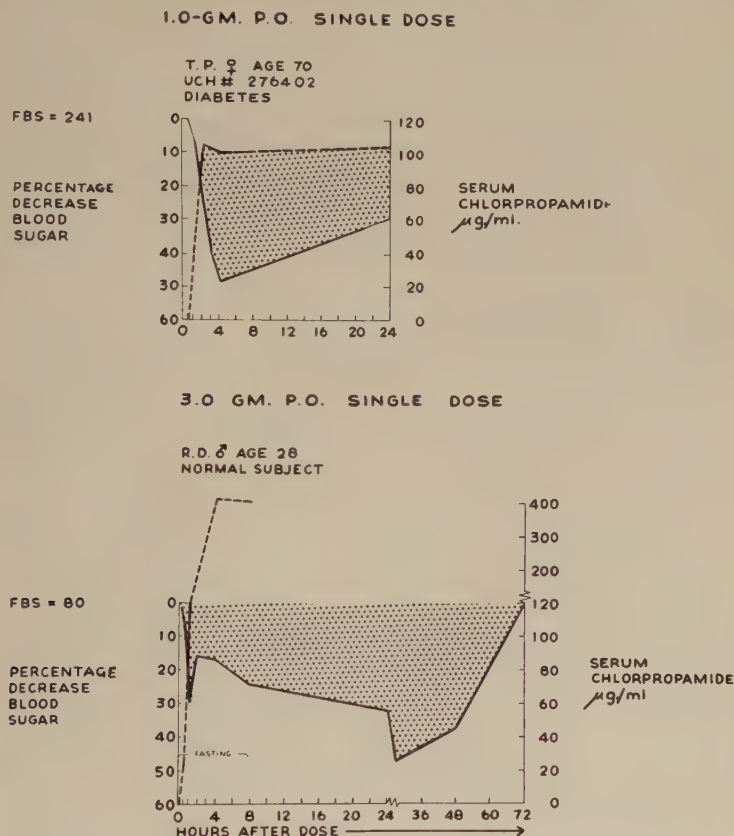


FIGURE 4. Serum levels of chlorpropamide compared to changes in blood sugar at the 1.0-gm. dose (upper) and 3.0-gm. dose (lower).

the total areas of blood sugar fall below the base line are compared. However, the difference appears to be due to the prolonged activity of chlorpropamide at this dosage inasmuch as chlorpropamide effects a decrease in blood sugar lasting as much as 72 hours, while tolbutamide produces a decrease in blood sugar below base line levels that lasts less than 48 hours.

It would appear that the intrinsic potency of each drug has reached a maximum at this dose level and that their hypoglycemic effects are equal, acutely. Their over-all hypoglycemic potency, however, varies with the differences in duration of activity.

Blood levels of chlorpropamide and its hypoglycemic effectiveness. The serum levels of chlorpropamide measured simultaneously with blood sugar levels (shown in FIGURE 4) follow the 1.0-gm. and 3.0-gm. single oral dose. It may be noted that the rate of increase and levels achieved for the serum values of chlorpropamide are proportional to the dosage. These suggest that absorption is rapid.

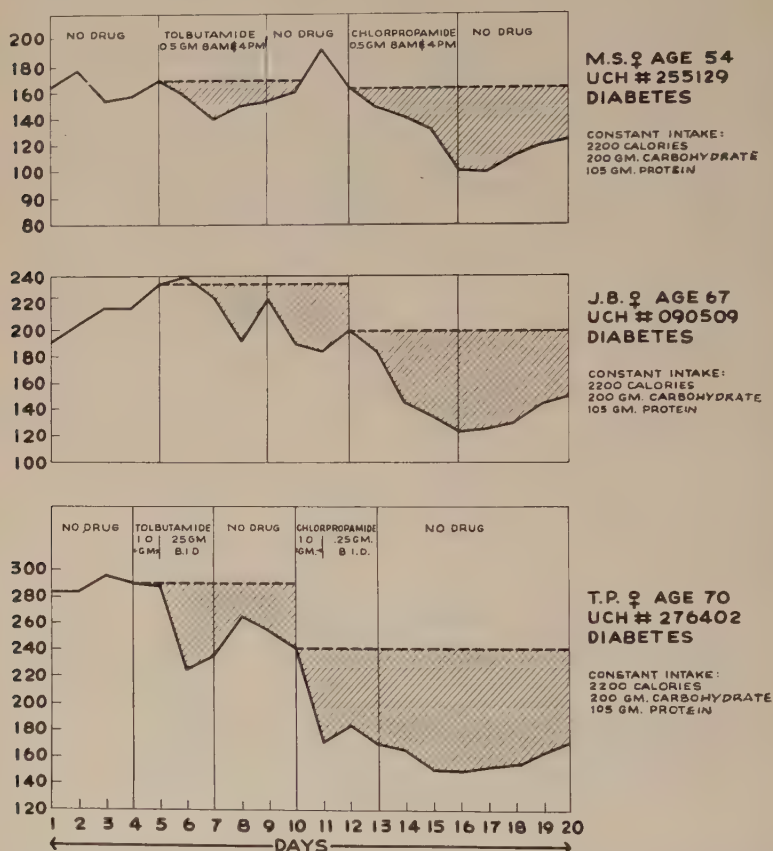


FIGURE 5. Comparative chronic hypoglycemic potency of chlorpropamide and tolbutamide compared at similar dose levels and time intervals.

Effective serum levels for significant acute hypoglycemic effect are also suggested in FIGURE 4. Assuming a fall in blood sugar greater than 25 per cent as significant, then serum levels of chlorpropamide necessary to achieve this degree of fall occur between 40 and 110 $\mu\text{g./ml.}$ with the 1.0-gm. dose, and between 20 and 120 $\mu\text{g./ml.}$ with the 3.0-gm. dose. These values are lower than similarly obtained plasma values for tolbutamide in 2 previous studies (80 to 180 $\mu\text{g./ml.}$ in the first study⁶ and 100 to 240 $\mu\text{g./ml.}$ in the second⁷). It would appear that the greater intrinsic potency of chlorpropamide is again

demonstrated by the fact that, as compared to tolbutamide, a lower serum value is required to achieve a significant hypoglycemic effect.

Chronic hypoglycemic effects. The comparative hypoglycemic potency upon repeated daily administration was studied in 3 hospitalized diabetic patients maintained on the metabolic ward on constant dietary intake for prolonged periods. FIGURE 5 demonstrates that the relative potency of chlorpropamide is again greater than that of tolbutamide in all of the individuals studied. Upon interruption of intake of each drug the hypoglycemic effect of the former persists longer, and fasting blood sugars remain below their original levels for 4 days. In patient T. P., 7 days after stopping the drug the fasting blood sugar levels had not returned to their previous base line levels. Twenty-four-hour urine sugars measured in these studies were generally below 100 mg. for 24 hours and were not changed significantly during therapy. The comparative areas of hypoglycemia reveal that the chronic potency of chlorpropamide upon repeated daily administration is of the order of 6 times that of tolbutamide.

Rate of disappearance of chlorpropamide. The apparent discrepancy between acute and chronic potency of chlorpropamide is most probably related to the prolonged rate of disappearance of this compound in the blood. Half-life periods were calculated based on the serum level of chlorpropamide following cessation of medication. TABLE 1 shows that the half-life rate of disappearance of chlorpropamide in 3 patients at different dose levels ranges from 36 to 39 hours. In 2 previous studies tolbutamide revealed a half life ranging from 4 to 6 hours as calculated from the disappearance of the compound from the plasma.^{6, 7} These figures are close to the sixfold increase in chronic potency for chlorpropamide compared to tolbutamide previously shown. In TABLE 1 we note that the serum level of chlorpropamide following repeated daily administration at doses of 0.5 gm. daily and 200 mg. 3 times daily remains steady during continued administration. It is apparent that the serum levels of chlorpropamide have risen to more than the effective levels previously determined to be between 20 and 120 $\mu\text{g.}/\text{ml.}$ In addition, the levels reach a plateau with repeated daily administration and remain relatively constant thereafter. Thus, after 72 and 107 days of treatment, respectively, in the two cases, serum levels remain between 250 and 350 $\mu\text{g.}/\text{ml.}$ throughout. Fasting blood sugar levels are not related to serum levels of chlorpropamide once the steady levels have been reached, although good control of hyperglycemia has been achieved in each case.

Mechanisms of action. The possible mechanisms of action of this new sulfonylurea compound appear to be similar to those of tolbutamide and the other sulfonylureas previously studied. Thus, in FIGURE 6 we note that the fasting blood sugar levels are not significantly affected in a depancreatized diabetic who received first saline and then chlorpropamide intravenously. This is in agreement with previous studies on other sulfonylurea compounds in animals⁸ and in man.⁹ Repeated daily administrations of chlorpropamide together with insulin for 4 days to this depancreatized patient failed to reveal any enhanced hypoglycemic effect of the insulin. There thus appears to be no inhibiting effect on the degradation of the insulin at the dosage employed.

TABLE 1
RELATIONSHIP OF ORAL DOSAGE TO SERUM LEVELS OF CHLORPROPAMIDE AND TO
BLOOD SUGAR LEVELS

Drug elimination							
Case	Subject	Dosage	Duration of therapy	Time after stopping dose, hours	Serum levels of chlorpropamide, $\mu\text{g./ml.}$	Calculated rate of disappearance of chlorpropamide (half life)	Fasting blood sugar
1	B. K. Normal	1.0 gm.	single dose	0	0	38 hours	80
				0.5	0		87
				1	31		55
				2	91		67
				4	114		104
				6	164		96
				8	137		68
				24	102		82
2	R. D. Normal	3.0 gm.	single dose	0	0	39 hours	80
				4	400		68
				8	400		57
				24	420		54
				32	205		43
				48	180		50
				72	112		92
3	J. B. Diabetes	0.5 gm. twice daily	start day 1 start day 2 start day 3 start day 4		0	36 hours	200
					67		182
					116		155
					187		135
				16	177		125
				60	69		130
				84	48		150
Chronic drug levels							
4	L. S. Diabetes	500 mg. daily	start day 1		0		200
			start day 11		350		138
			start day 23		250		88
			start day 45		320		118
			start day 74		260		130
			start day 107		252		99
5	P. P. Diabetes	200 mg. thrice daily	start day 1		0		242
			start day 4		280		133
			start day 32		320		94
			start day 51		250		81
			start day 72		310		125

The serum nonesterified fatty acids (NEFA) shown in the lower part of FIGURE 6 and measured simultaneously are not significantly affected following chlorpropamide. Thus, previously observed falls in NEFA upon glucose administration^{10, 11} do appear to be dependent upon insulin secretion and are not a direct effect of the sulfonylurea.

As a further means of comparing the action of chlorpropamide to that of tolbutamide, 3 unstable or "brittle" diabetics were given this hypoglycemic agent alone. All had a previous history of at least 1 episode of diabetic acidosis, with wide variations in daily levels of fasting blood sugars and in

their daily dosage of insulin. All 3 were found to be primary failures on chlorpropamide therapy at doses ranging from 0.5 gm. to 1.5 gm. daily, and demonstrated steadily increasing blood sugar levels and ketosis within the first 3 to 4 days of therapy. It is apparent, therefore, that functional beta cells are required for the action of chlorpropamide just as they are for the other sulfonylureas.

Finally, chlorpropamide was given in repeated daily doses to an adrenalectomized patient maintained on cortisone alone. In FIGURE 7 a rather exaggerated hypoglycemic response may be observed. The 24-hour urinary

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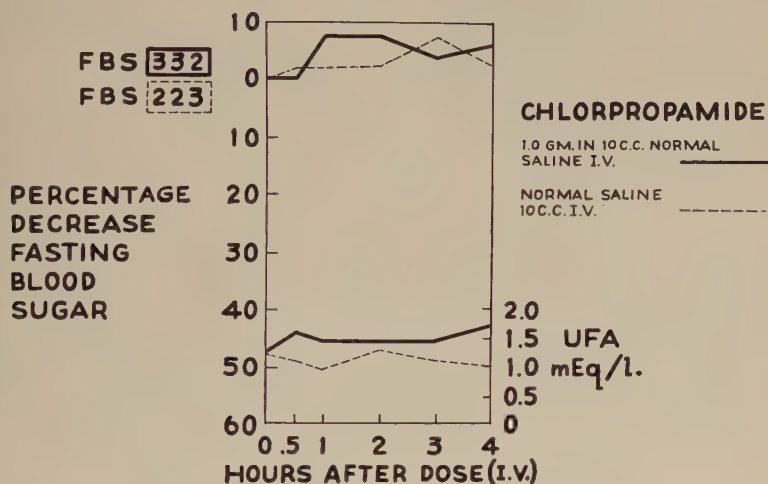


FIGURE 6. Acute effects of chlorpropamide (and a similar volume of saline given intravenously) on the fasting blood sugar and unesterified fatty acid levels of a depancreatized diabetic.

sugars shown in the lower part of the figure parallel the fasting blood sugars in onset and duration of fall. This suggests that chlorpropamide may have a depressant action on the gluconeogenesis induced by steroid administration, an important factor in this patient's hyperglycemia.

Outpatient trials. A limited number of stable diabetics was given chlorpropamide in the diabetes clinic. Criteria for selection of patients were the same as those used to select candidates for tolbutamide therapy. None had a previous history of acidosis or marked variations in blood sugar levels, and all had the onset of diabetes after the fourth decade. TABLE 2 summarizes the results. Thirteen such patients were tried on chlorpropamide. Two had been treated on dietary regimen alone without success. Three patients had been well controlled on small doses of insulin, and 8 patients had been on tolbutamide previously with good control and no untoward effects. It

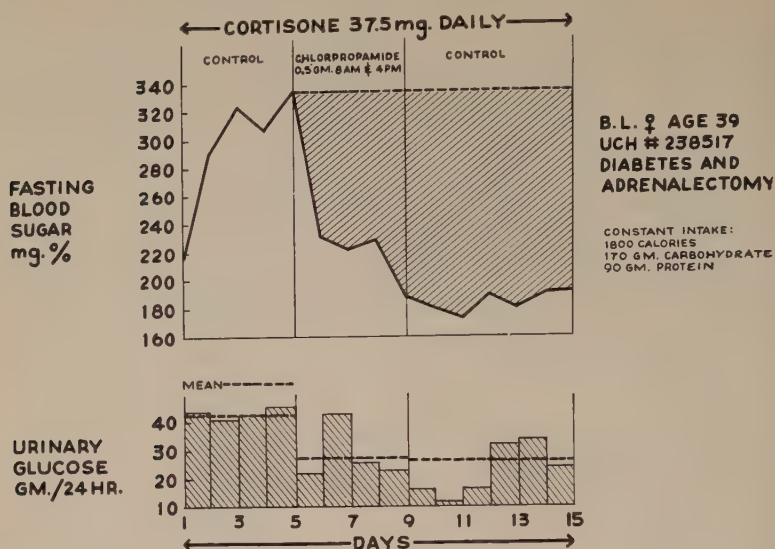


FIGURE 7. Increased sensitivity and prolonged hypoglycemic effect of chlorpropamide administration to an adrenalectomized diabetic patient.

was found that 7 patients complained of nausea soon after starting chlorpropamide. Doses in excess of 600 mg. daily were consistently associated with nausea; reducing the dosage below this level was generally followed by relief of symptoms. Four patients, however, had to be returned to their previous treatment because of continuing nausea. Nine patients now remain on chlorpropamide therapy and are doing well on doses ranging from 250 mg. to 600 mg. daily, with good control of hyperglycemia and glycosuria after

TABLE 2
OUTPATIENT TRIALS ON CHLORPROPAMIDE

Total number of patients.....	16
Unstable diabetics (all primary failures).....	3
<i>Stable diabetics</i>	13
Previous dietary treatment alone.....	2
Previous insulin.....	3
Previous tolbutamide.....	8
Nauseated at onset of treatment with chlorpropamide.....	7
Stopped treatment because of nausea.....	4
<i>Presently on chlorpropamide</i>	9
Previous dietary treatment alone.....	2
Previous insulin (15 U. NPH and 12 U. PZI).....	2
Previous tolbutamide.....	5
<i>Dosage, tolbutamide</i>	1 to 2 gm. daily
Control.....	good
Untoward symptoms.....	none
Duration of treatment: 6 months to 2 years	
<i>Dosage, chlorpropamide</i>	250 to 600 mg. daily
Control.....	good
Untoward symptoms.....	none
Duration of treatment: 3 months to 6 months	

from 3 to 6 months of treatment. Repeated hematologic, hepatic, and renal studies have revealed no toxic effects of the drug to date.

In our clinical experience the maintenance dose of chlorpropamide was found to be one-third to one-quarter that of tolbutamide. However, considering the chronic potency and the difference in the rate of disappearance from the blood, a relative dosage ratio of 1:5 or 1:6 should prove effective; further clinical studies may show this to be so.

Discussion

The present study suggests that chlorpropamide is a significantly more potent hypoglycemic agent than tolbutamide, both in acute and chronic activity. The former appears to be determined merely by the intrinsic potency of the drug, which is approximately twice that of tolbutamide. The chronic hypoglycemic potency of chlorpropamide, approximately 5 to 6 times that of tolbutamide, would appear to be determined by the longer half life of the drug in body fluids in its active form. This was found to be 36 hours instead of the 6 hours for tolbutamide, and might thus account for the difference in chronic potency between the 2. It was interesting to note that the serum level of chlorpropamide reached a relatively steady plateau not exceeding 350 μ g. per ml. following prolonged periods of administration, at the dosage of 0.5 gm. daily.

As to the mechanism of action, chlorpropamide would appear to require the presence of functioning beta cells of the pancreas just as do the other sulfonylureas previously studied. Chlorpropamide did not effect a decrease in blood sugar in a depancreatized diabetic. Repeated daily administration of chlorpropamide together with insulin for 4 days in this patient revealed no enhanced hypoglycemic effect through decreasing the rate of degradation of insulin. This is in agreement with most studies and in conflict with previous ones showing that higher dosages of sulfonylureas than those necessary to produce hypoglycemia may inhibit insulin degradation.¹²

A slightly exaggerated hypoglycemic effect observed in an adrenalectomized patient on cortisone therapy, suggests that chlorpropamide may also be an inhibitor of action on gluconeogenesis.

The maintenance dosage of chlorpropamide appears to lie between one-third and one-sixth that of tolbutamide. From our experience we conclude that if this is kept at less than 500 mg. per day, the incidence of nausea is markedly reduced.

Summary

Chlorpropamide has approximately twice the hypoglycemic activity of tolbutamide in its acute effect for a four-hour period.

Chlorpropamide shows approximately six times the chronic hypoglycemic activity of tolbutamide in experiments extending a number of days under strictly constant dietary intake. The half life of chlorpropamide appears to be six times greater than that of tolbutamide.

The mode of action of the two sulfonylurea derivatives appears to be identical.

The prolonged action and tendency to nausea, when 500 mg. daily is exceeded, suggest that care must be observed in its clinical use.

Acknowledgments

We acknowledge the cooperation of Herbert C. Moffitt, Jr., chief of the diabetes clinic of the University of California Department of Medicine; Robert Reiss; and other members of the diabetes clinic. We are indebted to Richard Havel for the studies mentioned under *Methods*.

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EXPERIENCES WITH CHLORPROPAMIDE WITH REFERENCE TO ITS INTERMITTENT USE

Farid Fuleihan and Calvin H. Plimpton

Department of Medicine, American University of Beirut, Lebanon

Our experiences with chlorpropamide were obtained in the Middle East where there are two reasons for a tremendous need for an oral antidiabetic preparation. First, the standards of hygiene and the habits of living make the use of insulin difficult, if not hazardous. Second, the people are accustomed to a gigantic consumption of carbohydrate. Arabic bread is indeed the staff of life, and carbohydrate is consumed at the rate of 500 to 800 gm./day. If the production of other foodstuffs were feasible from an economic and cultural point of view, many diabetics might be controlled by weight reduction and a shift in the source of calories. This is not now possible; hence an oral antidiabetic agent is most valuable.

TABLE 1

BLOOD LEVELS OF CHLORPROPAMIDE* IN MG. PER CENT ON A DAILY ORAL INTAKE OF 1 GM. AND CESSATION ON 8TH DAY

Patient No.	Days											
	1	2	3	4	5	6	7	8	9	10	11	12
13	5.8	5.9	12.2	9.2	22	26	18	15.6	9	10	5	4
14	—	—	—	—	20	23	15	10	4.5	5.5	2.5	1.5
17	6.3	11	12	14	16	14	13	11	12	6.8	—	2.5

* Supplied by Max Gahwyler of Chas. Pfizer & Co., Inc., Brooklyn, N. Y.

Chlorpropamide has certain characteristics that, apart from its hypoglycemic qualities, make it of special use in the management of diabetes. Upon the daily oral administration of a fixed amount, the blood level rises gradually to a maximum in 5 to 7 days. If the administration is then interrupted, the blood level falls very slowly so that there may be a concentration of 4 to 5 mg. per cent even after 5 days. This is illustrated in the figures furnished by Gahwyler on blood levels (TABLE 1). This suggested the possibility that the drug could be given intermittently.

Our patients were admitted to the hospital, where diet and activity could be controlled. The patients selected the diet they thought they could maintain for the period of study. Twenty-four hour excretion of urine sugar and both fasting and nonfasting blood sugars were followed. We were unable to do blood level determinations of chlorpropamide. There was no change in serial chemical evaluation of liver or renal function. No toxic effects were noted.

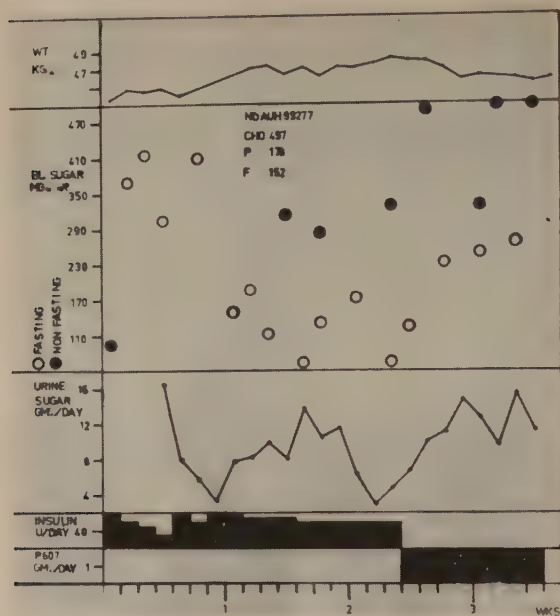


FIGURE 1. Unstable juvenile diabetic.

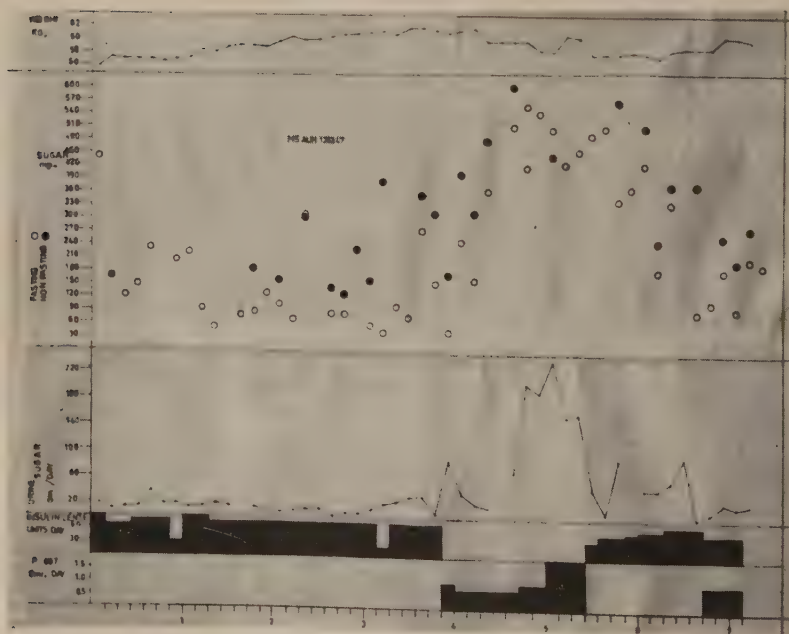


FIGURE 2. Severe diabetic with renal glycosuria.

Our detailed studies with chlorpropamide are illustrated by the following cases.

The first patient was an 18-year old girl (N. D. 99277) with diabetes of 7 months' duration; chlorpropamide did not control her diabetes in any way (FIGURE 1).

The second patient was a 50-year old farmer (M. S. 126347) with diabetes of 4 years' duration and a low renal threshold for sugar. His diabetes was severe, and again could not be controlled by chlorpropamide (FIGURE 2).

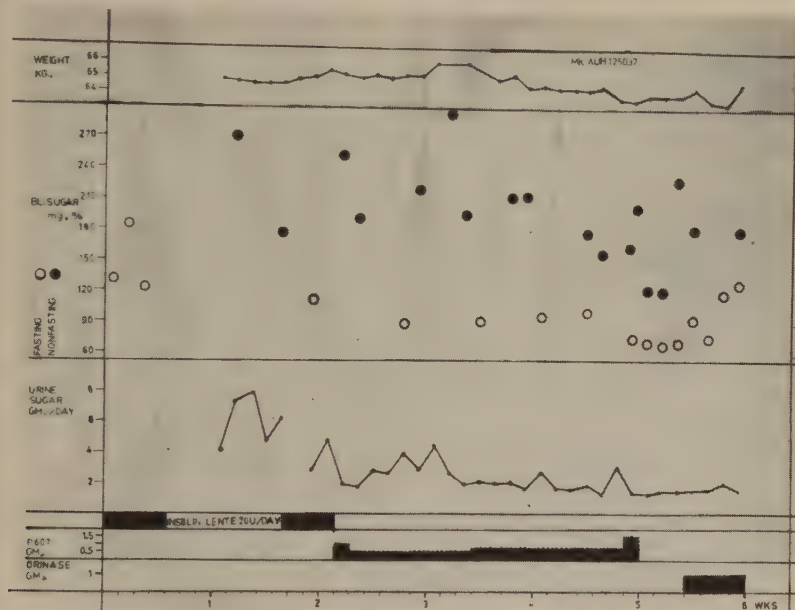


FIGURE 3. Mild diabetic.

It was interesting to note that there was no apparent effect on his renal glycosuria. The concomitant use of chlorpropamide with insulin did not appear to reduce the insulin dosage substantially.

The third patient was a 60-year old, a former wrestler (M. K. 125037) with mild diabetes for four years (FIGURE 3). He had been taking 20 units of insulin irregularly. He had proteinuria, slight edema, and a slightly low serum albumin of 2.9 gm. per cent. After a period of stabilization on insulin he was changed to chlorpropamide. On a daily maintenance dose there was no evidence of hypoglycemia, but the interesting feature was that a single dose of 1.5 gm. maintained his blood sugar and 24-hour urine excretion within normal limits for 72 hours, and without hypoglycemia (FIGURE 4). There was no evidence of acceleration of the diabetic vascular damage while on chlorpropamide. His eyegrounds, proteinuria, and hypoalbuminemia remained constant. His urea nitrogen fluctuated between 15 and 27 mg. per cent.

The fourth patient was a 50-year old man (K. D. 89351) with diabetes of

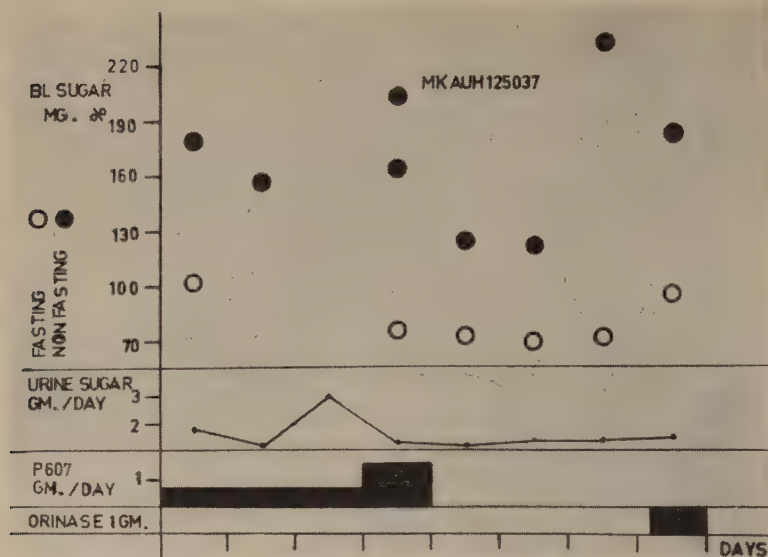


FIGURE 4. Mild diabetic with prolonged response to chlorpropamide.

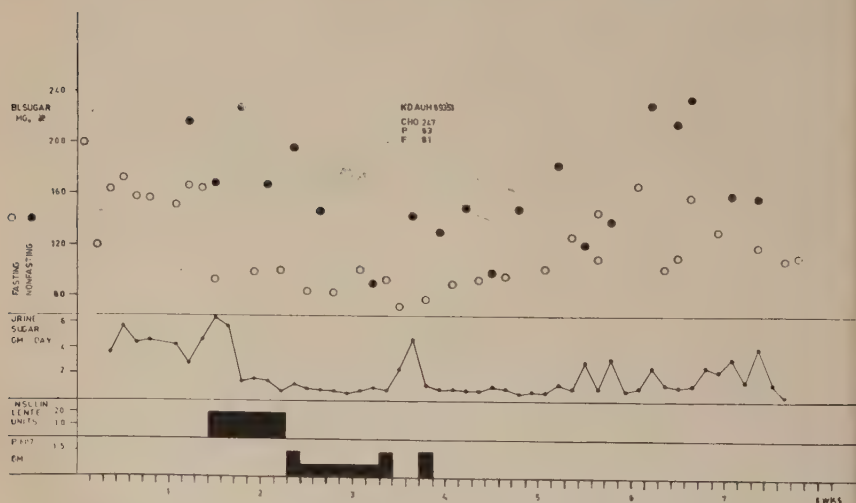


FIGURE 5. Mild diabetic with prolonged response to chlorpropamide.

2 years' duration. He was a mild diabetic and of a comfortable, middle-aged habitude that resists all changes in weight (FIGURE 5). On his particular diet the need for 20 units of insulin is demonstrated, and it is apparent that very small doses of chlorpropamide given at considerable intervals controlled his blood and urine sugar.

The fifth patient was a 60-year-old woman with diabetes of four months

duration. Difficulties in her control by intermittent chlorpropamide are shown in FIGURE 6.

The studies have been extended to include our clinic patients whose diet and activity cannot be controlled. In five patients taking 0.5 gm. of chlorpropamide every two to three days, the nonfasting blood sugar could frequently be maintained at the upper limits of normal. However, this was much less striking than in the third patient (M. K. 125037), whose 72-hour control probably represented the delayed excretion of a cumulative dose. On

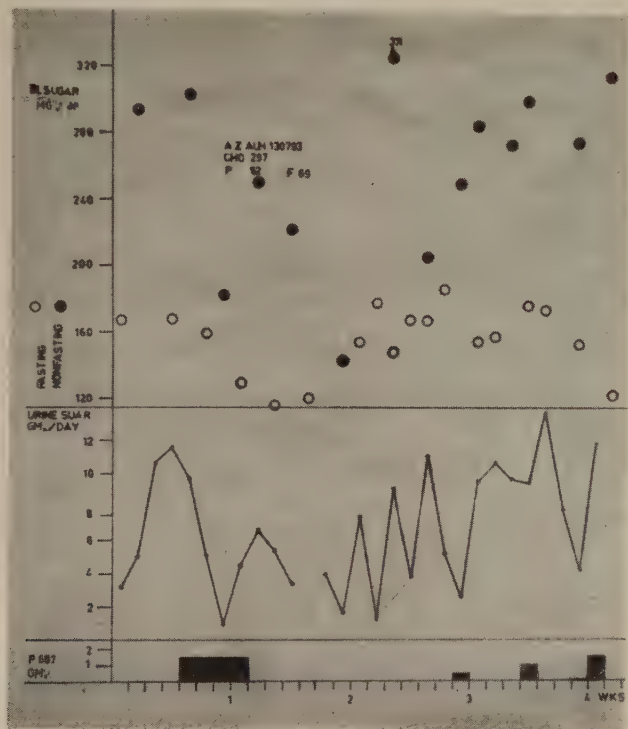


FIGURE 6. Mild diabetic with inadequate response to intermittent chlorpropamide.

the other hand, our fourth patient (K. D. 89351) maintained normal values for a very long time after a relatively small dose of chlorpropamide. The fifth patient represents some of the difficulties inherent in attempting intermittent dosage.

We have not had the opportunity to study the specific effects of this drug on the complications of diabetes. In the cases studied, there was no evidence for any acceleration of the degenerative processes in retinal, coronary, or renal circulations.

The intriguing aspect of chlorpropamide is its mechanism of action: particularly, how this drug can, while circulating at slowly decreasing blood levels, maintain the blood sugar within normal limits despite three ingestions of carbohydrate a day.

Conclusion

In occasional patients the slow excretion of chlorpropamide may permit its intermittent use.

Discussion of the Paper

J. D. N. NABARRO (*Middlesex Hospital, London, England*): From the purely clinical standpoint, the question that must be answered is: Does chlorpropamide have any advantages over tolbutamide? Chlorpropamide is certainly easier for the patient to take, and it can be given once daily, whereas tolbutamide must be given in divided doses.

A major problem with tolbutamide at the present time is the development of acquired or secondary resistance to its hypoglycemic action. This is not the same as secondary failure, a term used by several contributors to this monograph. Secondary failure is more often due to the fact that the patient has broken his diet than to acquired resistance to the hypoglycemic drug, and it is not reasonable to blame the drug for the indiscretions of the patient. Acquired resistance can be established only if the patient is hospitalized and remains hyperglycemic while on a strict diet or if a high dosage of tolbutamide (3 gm./day) can be withdrawn without any further rise of blood sugar. Our incidence of acquired resistance is 10 per cent for patients on the drug for more than 6 months, and it has developed between the seventh and seventeenth months of treatment. One patient with acquired resistance was transferred to chlorpropamide without adequate response.

We have had to withdraw tolbutamide in 7 per cent of our patients because of side effects: 4 per cent abdominal and 3 per cent skin changes. One patient who was unable to take 1.5 gm. of tolbutamide because of abdominal discomfort is now well controlled without symptoms on 0.25 gm. of chlorpropamide daily.

One final point that I should like to make is that we must not underestimate the length of time required for proper assessment of the value of these drugs. We have recently examined all our data on tolbutamide-treated patients and have found that the percentage still on the drug and in satisfactory control drops steadily over the first 18 months. However, between 18 months and 3 years the figure levels out at about 35 per cent of those giving a good initial response. This, of course, tells us nothing about the antidiabetic value of the drug, and it relates only to its hypoglycemic action.

A CLINICAL AND PHARMACOLOGICAL COMPARISON OF CHLORPROPAMIDE AND OTHER SULFONYLUREAS*

J. M. Stowers, L. W. Constable

Department of Medicine, University of St. Andrews, Queen's College, Dundee, Scotland

R. B. Hunter

Department of Pharmacology and Therapeutics, University of St. Andrews, Queen's College, Dundee, Scotland

The effective blood level of chlorpropamide† probably lies between 4 and 16 mg. per 100 ml., which is comparable with those of carbutamide and tolbutamide, but chlorpropamide differs significantly from tolbutamide in that it is not readily metabolized to a physiologically inactive compound as tolbutamide is to carboxytolbutamide.¹ In fact, the ultraviolet absorption spectrum of chlorpropamide in the blood seems to be identical with that of the drug in the urine, suggesting that it has undergone no chemical change.

TABLE 1
MEAN HALF LIFE AND PERCENTAGE OF BODY WEIGHT IN WHICH THREE
SULFONYLUREAS WERE DISTRIBUTED

	Half life hours	Volume of distribution as percentage of body weight
Carbutamide.....	40 (8)	approx. 45
Tolbutamide.....	3.5 (6)	approx. 20
Chlorpropamide.....	34.5 (8)	approx. 18

Numbers of individuals in parentheses.

This effective blood level of chlorpropamide can be maintained with as little as 0.5 gm./day in contrast to the three- or fourfold dosage that is required for tolbutamide. Both drugs are rapidly absorbed: they produce peak serum levels 2 to 3 hours after ingestion. The volume of distribution of both drugs in the body is similar (TABLE 1) and approximates the volume of the extracellular fluid, whereas carbutamide is distributed in about twice as large a volume. The "half life" of chlorpropamide, that is, the time required for any given serum level to fall by 50 per cent, is approximately 10 times longer than that of tolbutamide and nearly as long as that of carbutamide (TABLE 1). FIGURE 1 illustrates how these data were obtained. A single dose, for example 2 gm. of a sulfonylurea, is given to an individual who has not previously received this drug. Blood samples are then withdrawn at intervals determined by the anticipated half life, and the concentrations of the drug are estimated in these samples, using a controlled serum taken before the experi-

* The work reported in this paper was supported in part by a grant from the Scottish Hospital Endowments Research Trust, Edinburgh, Scotland.

† Supplied by R. H. Gosling of Pfizers Ltd., Folkestone, England.

ment started. When these concentrations are plotted on a log scale against time, a straight line is obtained. This line can then be extrapolated back to zero time, and the theoretical initial concentration is then read from the scale. The assumption is made that all the drug administered was absorbed and that no significant fraction was excreted before absorption was complete. Having thus derived the theoretical initial concentration, the time at which

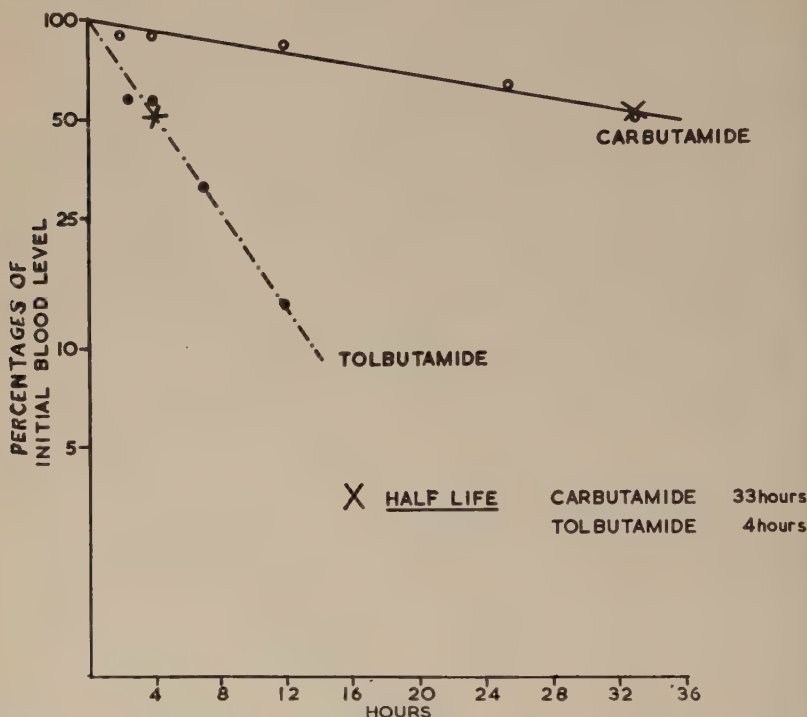


FIGURE 1. Rate of fall in serum level of carbutamide and tolbutamide after a single administration of 3 gm. of each to the same individual at different times. The theoretical initial concentration is obtained by extrapolating the line representing the rate of decrease of blood levels recorded on a log percentage scale. Reproduced by permission of *The Lancet*.

it will have fallen by 50 per cent can be read from the graph. In the example illustrated the concentrations are expressed as percentages of the initial blood level so that the half lives of carbutamide and tolbutamide fall on the same horizontal line.

The volume of distribution of the drug can readily be calculated provided the theoretical initial concentration and the size of the dose administered are known, again assuming that absorption was complete.

If it is accepted that chlorpropamide is distributed in the body in a volume corresponding to that of the extracellular fluid, it is of interest to know whether it is freely in solution or bound to protein. There is some evidence that it is protein-bound because serum containing 15 mg./100 ml. of chlor-

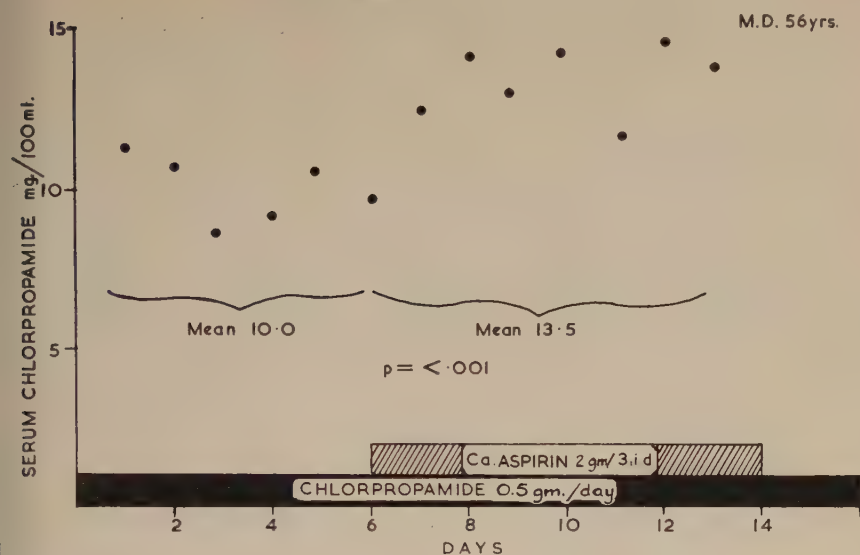


FIGURE 2. Effect of salicylate on chlorpropamide serum level.

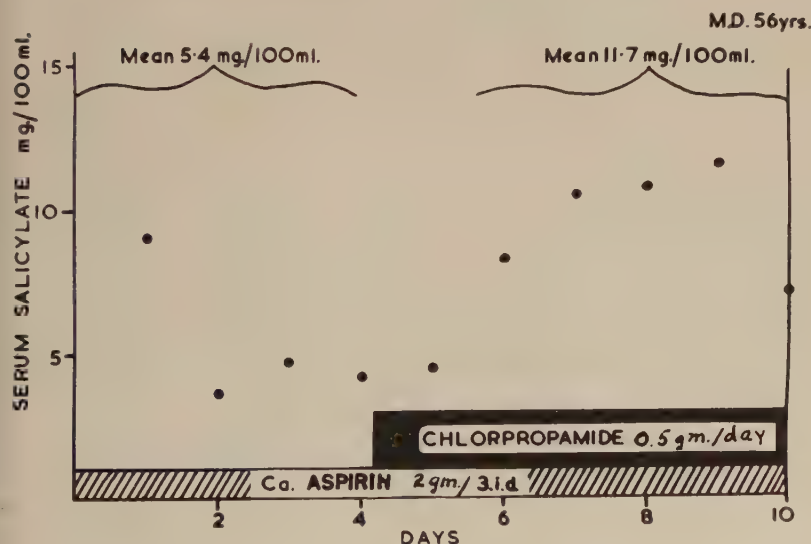


FIGURE 3. Effect of chlorpropamide on serum salicylate level.

propamide had all traces of chlorpropamide removed by the precipitation of the protein with trichloroacetic acid, and neither chlorpropamide nor tolbutamide has been found in the cerebrospinal fluid when the serum concentration has been as high as 14 to 24 mg./100 ml. The protein-binding is evidently a loose binding because chlorpropamide can be dialyzed out of serum quite readily. The difference in the half lives of tolbutamide and

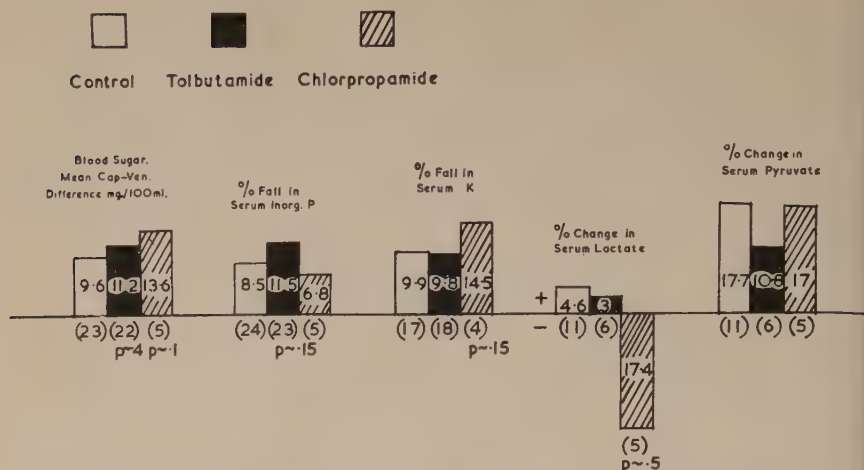


FIGURE 4. Changes during glucose tolerance tests (50 gm. *per os*) in mild diabetics. Numbers are shown in brackets.

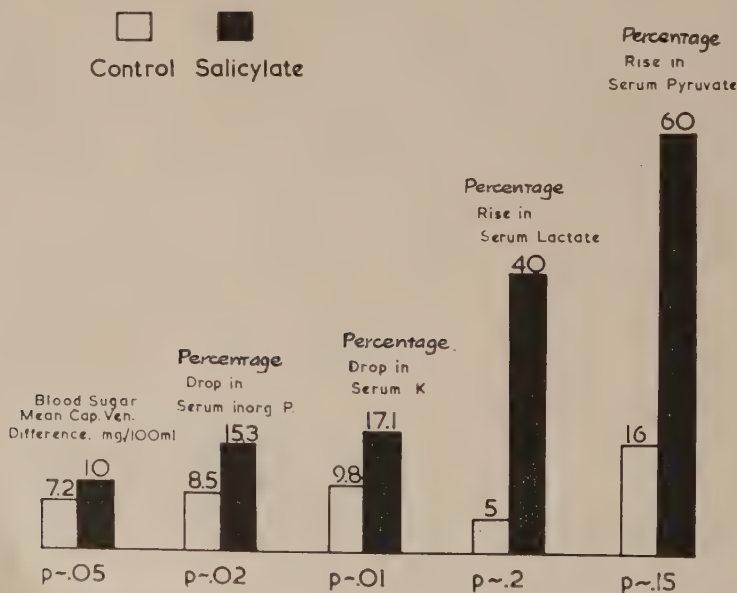


FIGURE 5. Changes during glucose tolerance tests (50 gm. *per os*) in 11 mild diabetics.

chlorpropamide must depend largely on their handling by the kidneys, since the urine is the vehicle of their elimination. The protein-binding itself would tend to limit the renal excretion of those drugs, but the mechanism of tubular transport must be the key to the understanding of this problem. Some interesting sidelights were thrown on it by studying the interaction of chlorpropamide and salicylates² on blood levels, first when given singly and

then when given together to the same individual (FIGURES 2 and 3). Such results are explained most easily by mutual interference with some process of tubular secretion in the kidney, and may have some practical bearing on therapy, since their hypoglycemic effects can be shown to be additive.

In comparing the hypoglycemic potency of tolbutamide and chlorpropamide, the superiority of chlorpropamide is apparent, notwithstanding the small number of cases that we have properly studied (TABLE 2). At blood levels that were slightly lower, chlorpropamide produced a 61 per cent fall in the fasting venous blood sugar compared with a 29 per cent fall induced by tolbutamide. Indeed, so great is the hypoglycemic potency of chlorpropamide that we have twice been troubled by hypoglycemia induced in mild diabetics, a feature we have rarely experienced with tolbutamide. Unlike

TABLE 2
HYPOGLYCEMIC EFFECT OF TOLBUTAMIDE AND CHLORPROPAMIDE
Mild diabetics (numbers in parentheses)

	Mean percentage fall in fasting blood sugar		Mean drug level mg./100 ml.
	Drug day	No treatment day	
Tolbutamide.....	29	(25)	12.4
Chlorpropamide.....	61	(5)	11.4
	$p \sim 0.005$		

our experience with tolbutamide, we have never found chlorpropamide to be even probably responsible for dyspepsia. We have had three patients who had had dyspepsia, one an actual case of hematemesis that we attributed to tolbutamide. All three were changed over to chlorpropamide and made no further complaints about their digestion. The less frequent administration of chlorpropamide in itself is no doubt helpful in this respect.

Data have been obtained on patients taking tolbutamide and chlorpropamide during oral glucose tolerance tests and compared with similar data obtained when they received no treatment. In this way it was hoped that some information might be gained regarding the method of the hypoglycemic action shown. The parameters measured were the differences between the capillary and venous blood sugar levels,³ the fall in the serum inorganic phosphate⁴ and potassium,* and the change in the serum level of lactate⁵ and pyruvate,⁶ all estimated six times during each glucose tolerance test. The results are expressed in FIGURE 4.

If there had been an insulinlike action in the peripheral tissues, characteristic changes would have been found in each of these different measurements. In fact, no significant changes were observed and thus no evidence was obtained that these sulfonylureas acted like insulin in the muscles. The fact

* Internal standard flame photometry.

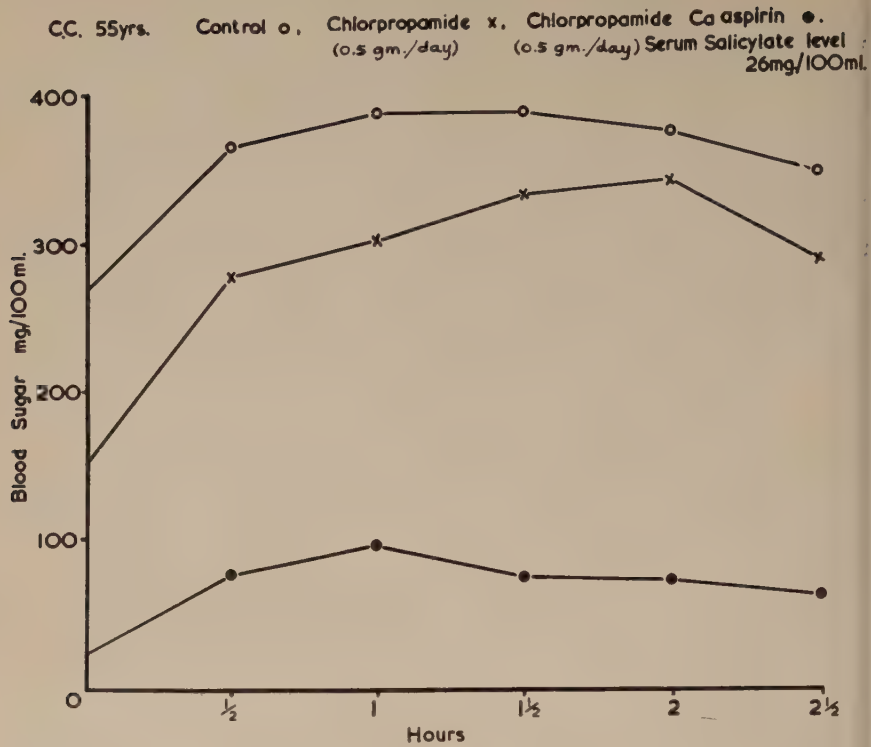


FIGURE 6. Glucose tolerance curve obtained in the same patient when on no treatment, when receiving chlorpropamide (one-half gm./day), and when receiving the same dose of chlorpropamide with calcium aspirin sufficient to bring the serum salicylate level to 26 mg./100 ml.

that relatively they have much more effect on the fasting than on the post-absorptive blood sugar levels suggests that they inhibit glucose output by the liver. As a comparison the same type of data are shown for salicylates (FIGURE 5). With salicylates all the changes, several of them statistically significant, are in the direction that would be expected if salicylates were

TABLE 3
BLOOD GLUCOSE LEVELS IN 4 MILD DIABETICS
Glucose Tolerance Tests, 50 gm. *per os*

Patient.....	Fasting venous blood glucose mg./100 ml.				Peak venous blood glucose mg./100 ml.			
	M.D.	M.K.	H.C.	C.C.	M.D.	M.K.	H.C.	C.C.
Control.....	248	180	167	269	343	323	269	385
Sulfonylurea.....	62	73	120	151	189	235	209	340
Ca aspirin.....	123	169	143		255	347	233	
Sulfonylurea + Ca aspirin....	94	45	46	22	203	297	129	96

acting like or by means of insulin. FIGURE 6 and TABLE 3 illustrate how salicylates sometimes can enhance the hypoglycemic effect of the sulfonylureas. The serum level of salicylates necessary to achieve this action is no higher than 10 mg./100 ml. in some of the cases we studied.

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ONE YEAR OF CLINICAL EXPERIENCE WITH CHLORPROPAMIDE IN MORE THAN ONE HUNDRED DIABETIC PATIENTS

Zeb L. Burrell, Jr., Alberto Martinez, Lenette O. Burrell

Department of Medicine, Milledgeville State Hospital, Milledgeville, Ga.

During the past 13 months we have treated 130 diabetic patients with chlorpropamide, the duration of treatment varying from 1 to 13 months and averaging 6.3 months.

Method

Our only criterion for selection of patients for a trial of chlorpropamide therapy was the presence of diabetes in a reasonably cooperative patient. The presence of common diabetic vascular complications did not preclude a trial of this drug unless they were acute, nor did the occurrence of vascular complications result in a change in therapy as long as the patient remained able to take the drug. Chlorpropamide therapy was never begun while the patient was clinically acidotic; however, the drug was tried later on several patients who were acidotic when first seen. The majority of the patients were hospitalized for initial therapy.

Most patients were started on the following therapy, with minor variations in individual cases. If the patient was on a long-acting insulin, this was discontinued abruptly, and he was started on 0.5 gm. of chlorpropamide daily, usually in divided doses. During the initial period of treatment the more acute cases were given regular insulin, as indicated by glycosuria. Revisions of chlorpropamide dosage were made upward or downward as necessary, 5 to 7 days usually being allowed before revision upward due to the accumulation noted in the use of this drug. The dosage was gradually increased to tolerance or adequate control, this being normoglycemia 75 per cent of the time and the absence of significant symptoms or side effects.

We have attempted combined therapy on but one patient. No attempt has been made to evaluate this form of treatment.

Urine analysis, liver function, and hematological studies, as well as blood sugar determinations, were followed frequently during the initial treatment and approximately once monthly on chronic treatment.

Results

Before analyzing our results it is necessary to comment on the patients who compose our series. Approximately two thirds of our patients were inmates of a state mental institution. As a group these patients constitute a poor risk for any study. There are a number of reasons for this, including advanced age, the average age of our entire series being 61.1 years. Most of these patients have mental deterioration that seriously limits their ability to cooperate in such matters as diet. The mental changes in many of these patients have been due to previous vascular disease of the central nervous

system, renal insufficiency, or uncontrolled diabetes itself. Many of these patients have had previous frank episodes of cerebral, myocardial, or peripheral vascular insufficiency.

Of 130 patients, 108 (83 per cent) were considered adequately controlled on chlorpropamide; 83 (64 per cent) are still under study. In diabetics previously on less than 60 to 80 units of insulin per day there was a small correlation between prior insulin dosage and likelihood of control with this drug alone (TABLE 1). There was a small but significant correlation between

TABLE 1
CORRELATION OF PRIOR TREATMENT WITH RESPONSE TO CHLORPROPAMIDE*

Prior therapy	Total cases	Response to chlorpropamide	
		Adequate control	Inadequate control
No treatment.....	17	17	0
Insulin:			
0 to 20 units.....	23	21	2
21 to 40 units.....	43	36	7
41 to 60 units.....	19	16	3
61 to 80 units.....	9	4	5
Over 80 units.....	9	6	3
Unknown amount.....	7	5	2
Tolbutamide:			
0 to 1.0 gm.....	9	9	0
1.5 to 2.0 gm.....	7	5	2
Over 2.0 gm.....	3	2	1
Total.....	146	121	25

* Sixteen patients received both insulin and tolbutamide.

duration of diabetes and probability of control. After a duration of 10 years the patient was less likely to respond to chlorpropamide with adequate control (TABLE 2). There was a slight but positive correlation between age at the time of onset and probability of control, the older patient being the better candidate (TABLE 3). One hundred and eleven of our patients were above 40 years of age at onset of diabetes, and 86 per cent were adequately controlled. Only 2 of our patients were juvenile diabetics, and both of these cases failed to respond.

Of 4 patients previously poorly controlled on tolbutamide, 3 showed improved control on chlorpropamide. Only 4 patients poorly controlled on chlorpropamide were given a trial of tolbutamide, which also failed. All 12 patients previously controlled satisfactorily with tolbutamide were also adequately managed with chlorpropamide.

No extensive comparative studies between tolbutamide and chlorpropamide have been undertaken. However, the following three observations appear reliable. First, chlorpropamide is about 3 times more potent than

tolbutamide on a weight-to-weight basis. Second, hypoglycemia can be produced with chlorpropamide in most patients; this is uncommon with tolbutamide. Finally, the onset of action of chlorpropamide is approximately 50 per cent slower than with tolbutamide; however, the observed cumulative effect with chlorpropamide is more lasting than with tolbutamide. This effect has been noted for as long as 7 days following the discontinuance of chlorpropamide.

TABLE 2
CORRELATION OF DURATION OF DIABETES WITH RESPONSE TO CHLORPROPAMIDE

Duration of diabetes (years)	Total cases	Response to chlorpropamide	
		Adequate control	Inadequate control
0 to 1	20	20	0
1 to 2	21	19	2
3 to 5	42	34	8
6 to 10	36	28	8
11 to 20	9	6	3
20	2	1	1
Total.....	130	108	22

TABLE 3
CORRELATION OF AGE AT TIME OF ONSET WITH RESPONSE TO CHLORPROPAMIDE

Age at time of onset (years)	Total cases	Response to chlorpropamide	
		Adequate control	Inadequate control
0 to 20	2		2
21 to 40	17	12	5
41 to 80	111	96	15
Total.....	130	108	22

After initial control there appeared to be a gradual reduction in the dosage required for adequate management. It has been possible to discontinue the drug entirely in 5 patients after initial control; this could represent spontaneous or induced remission. At the time these patients were admitted to the series, treatment in addition to diet appeared to be indicated. Four of these patients had been on the following treatments: 60 units NPH insulin daily for one year; 30 units NPH insulin daily for 6 years; 15 units NPH insu-

lin for 12 years followed by tolbutamide (0.5 gm.) for 6 months; and tolbutamide (3 gm./day) for 8 months. The fifth patient was a newly discovered untreated diabetic. These patients will be watched with interest.

Forty-six per cent of our patients on maintenance therapy require 250 mg. or less of chlorpropamide daily. Seventeen per cent require 250 to 500 mg./day; 33 per cent require 0.6 to 1.0 gm./day, and only 4 per cent require more than 1 gm./day.

In our series there were no untoward hematological effects; several of our patients had mild anemias both prior to and during treatment. These conditions did not seem to be affected by the drug. Low normal white cell counts were seen with some frequency, rarely dropping below this level. There was no instance of agranulocytosis. One patient had a mild chronic lymphatic leukemia that was present prior to treatment and asymptomatic.

There were no significant renal effects. Many of the patients had albuminuria both prior to and during treatment. Most of these were thought to be manifestations of diabetic nephropathy, and were apparently unchanged by therapy.

Weakness unrelated to hypoglycemia was a complaint of several patients early in the course of the treatment. This was not of such severity as to require discontinuance, and subsided in a few days.

One patient developed a jaundice that was thought to be induced by chlorpropamide. This individual developed a rather severe skin reaction and eosinophilia. The drug was discontinued because of the skin reaction; jaundice was apparent the next day. Laboratory studies suggested primarily obstructive type jaundice. Both jaundice and skin reaction cleared within a month after the drug was withdrawn. After 3 challenge doses of 0.25 gm./day there was a dermatitis, and the drug was discontinued. Although it was unsubstantiated, we feel this was possibly a hypersensitivity type of hepatic reaction.

Three other patients developed jaundice while on chlorpropamide therapy. In one patient the drug effect was eliminated by surgical correction of an obstructing lesion; this patient has subsequently been maintained on treatment. In another patient the drug effect was eliminated by readministration and continued treatment. The third patient developed a jaundice associated with biliary tract disease and frank acute pancreatitis. The drug was discontinued during the period of treatment for pancreatitis. The pancreatitis and jaundice cleared completely, and the drug was resumed. The patient has remained clear for 2 months after chlorpropamide was resumed. Transient abnormal liver function studies, such as elevation of thymol turbidity, were noted in another 12 patients. We are unable to assess the significance of this, as we have no control studies in a similar group of diabetics on other forms of treatment.

The drug was discontinued in a total of 47 patients. Seventeen of the 47 patients had the onset of a terminal illness while under study. Autopsy was performed in those cases in which the cause of death was not clinically obvious. No death was thought to have been related to the drug. The causes of death were generally those expected in elderly diabetics. Nine

were from cerebral vascular thrombosis or hemorrhage, and there were 3 episodes of fatal pulmonary emboli. Two deaths were due to myocardial infarction with failure. There was 1 death due to each of the following: staphylococcic pneumonia with lung abscess, staphylococcic pneumonia, and diabetic nephropathy with uremia. Special attention was paid to the liver at autopsy in patients who were on the drug at time of death. No histological change was noted.

The drug was discontinued in 9 of the 47 patients due to poor control. Eight of these patients are labile diabetics and are also poorly controlled on their present insulin regime in spite of our best efforts in that direction. Seven patients were lost to follow-up. These essentially represented patients who manifested psychiatric recovery and were thereby discharged from the institution. As previously mentioned, it was possible to discontinue the drug in 5 patients because of apparent remission of the diabetic state. Gastrointestinal side effects, especially nausea and vomiting, necessitated discontinuance in 5 patients. Several other patients manifested lesser degrees of nausea, which gradually subsided with continuation of treatment.

Chlorpropamide was discontinued in 4 of the 47 cases due to severe skin reactions that did not subside on antihistaminic treatment and continuation of the drug. These reactions subsided on withdrawal of chlorpropamide, and the role of the drug was substantiated by a recurrence with a second course of the drug.

In addition to the 130 diabetic patients, 6 nondiabetic chronic schizophrenics were given 0.5 gm. chlorpropamide daily 4 days weekly for 6 weeks in limited metabolic studies. Four of these patients suffered early morning hypoglycemia in spite of midnight feedings. This was the only significant observation. No effect on the psychiatric state was noted.

Summary and Conclusions

(1) Chlorpropamide has been given to 130 diabetic patients for an average of 6.3 months; 83 per cent of these cases were adequately controlled on this medication.

(2) Side effects were those usually noted with drugs of the sulfonamide group and occurred in some 12 per cent, requiring discontinuance in 8 per cent. Nausea, vomiting, and skin reactions were the most commonly noted side effects. One patient manifested a jaundice possibly caused by hypersensitivity to the drug.

(3) Chlorpropamide is a potent hypoglycemic agent in a large percentage of nonjuvenile diabetic patients as well as nondiabetic individuals.

(4) We feel that chlorpropamide is a significant addition to our armamentarium in the treatment of diabetes mellitus.

THE CORRELATION BETWEEN ORAL DOSAGE, BLOOD LEVELS, AND CLINICAL AND METABOLIC ACTIVITY OF CHLORPROPAMIDE IN THE TREATMENT OF DIABETES MELLITUS*

Samuel B. Beaser

*Harvard Medical School, and Abraham Rudy Diabetes Clinic of the Medical Service,
Beth Israel Hospital, Boston, Mass.*

Chlorpropamide was administered over a period of 8 months to 60 adult diabetic patients. These were all private patients, among whom were a considerable number sufficiently reliable in their adherence to diet to be suitable for experimental trials. Treatment was combined with insulin and other hypoglycemic agents in 12 of the cases. Follow-up visits were made at frequent intervals, both blood sugar and blood chlorpropamide level determinations being made at each visit. The objective of the study was to determine the therapeutic effectiveness of chlorpropamide and to ascertain the relationship between dosage, blood levels, and clinical action.

Method

The patients had previously been treated with diet alone, with insulin, or with tolbutamide. Some had also been treated with carbutamide and DBI. As in the case of tolbutamide, the drug was used to replace all previous medication where possible. In two patients it was supplemented with insulin when necessary.

The patients were all on a nonweighed diabetic diet and returned at intervals of 3 days to 1 month. The blood sugar level was determined 1 hour after breakfast or lunch on each occasion by a picric acid method that yields results equivalent to those of the Folin-Wu test. Blood chlorpropamide levels were determined by a spectrophotometric method.^{†1} The dose of chlorpropamide was deliberately varied widely in a large number of patients, usually over the range of 50 mg. to 600 mg./day, but in a few instances up to 1.5 gm./day. The total amount was administered as a single dose after the evening meal. In some instances the drug was omitted for trial periods.

Of the 60 patients, 34 had enough multiple determinations of blood chlorpropamide levels to form the basis for an analysis of the relationship between oral dosage and blood level of the drug. In all, 214 determinations were obtained for this purpose, each after at least one week or more at a given dosage level.

A composite curve of all the determinations (FIGURE 1) shows a direct proportion between dosage and blood level of chlorpropamide. The regression curve shows that for the range of dosage between 50 mg. and 1000 mg./day, each 100 mg. increase in dosage of the drug raises the blood level

* The work reported in this paper was supported by the Clinical Research Department, Chas. Pfizer & Co., Inc., Brooklyn, N. Y.

† Developed by the Chemical Research Department of Chas. Pfizer & Co., Inc.

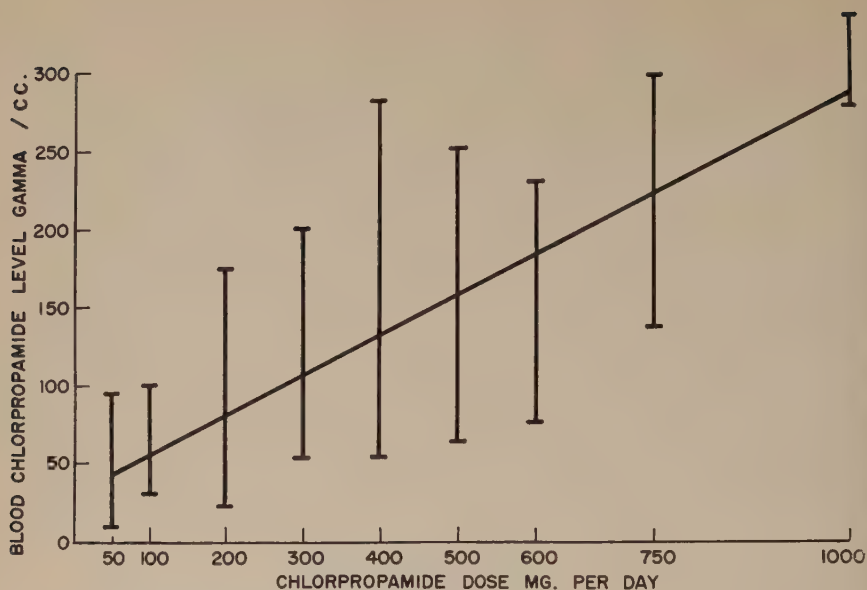


FIGURE 1. Regression curve of relationship between dose and blood levels of chlorpropamide: 34 diabetic patients, 214 determinations.

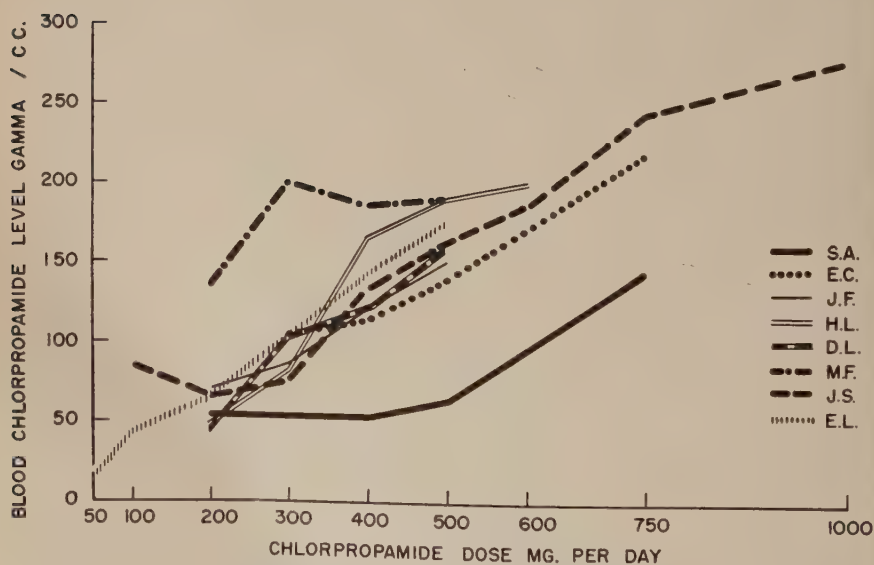


FIGURE 2. Individual patient blood chlorpropamide responses to oral therapy: 8 patients at 4 dosage levels.

by roughly 25 gamma/cc. In FIGURE 2, where the individual responses for 8 patients have been plotted for 4 different dosage levels, the patients show a large individual variability in the handling of the drug, although the proportional rise in blood level with dose still obtains. Striking variations occurred in 3 patients not illustrated, 2 with unusually low and 1 with high blood chlorpropamide responses.

The variability of the blood chlorpropamide levels was analyzed in 10 patients with 14 different patient-dosage levels. The doses studied varied



FIGURE 3. Diabetic patient L. D.: acute effect of increasing dosage of chlorpropamide upon blood sugar and chlorpropamide levels over a 9-day period.

from 0.2 to 1.0 gm./day, and there were 4 to 10 blood determinations at each dosage level over a period of 1 to 5 months. The variability of blood chlorpropamide levels between patients was obviously greater than those for a given patient at a given dosage level. In the latter, the blood levels varied over only a limited range for long periods of time.

The wide variation in dosage of chlorpropamide administered to some 25 diabetics made possible an analysis of the relationship between the dose of drug and pharmacological and clinical effect. This was done especially for the purpose of determining the area of dose-dependency of the drug. The diabetics studied were usually those of moderate severity (requiring up to 40 units of insulin daily), in whom the needed insulin could be replaced completely by chlorpropamide.

FIGURE 3 illustrates a subacute experiment. A 64-year-old white female (L.D.) with diabetes of 10 years duration had previously been regulated on tolbutamide alone (1.0 gm./day). Under hospital conditions, with fixed

diabetic diet, chlorpropamide was given in increasing doses over a 9-day period. There is a striking effect on blood sugar, with rising dose and blood level of chlorpropamide. While such an acute experiment is interesting, slower increments of dosage are necessary to establish quantitative relationships between dose, blood level, and hypoglycemic effectiveness.

Accordingly, the remaining patients were studied over periods of months with slow stepwise changes in the dose of the drug. As in the case of the other sulfonylureas, the end results of replacement therapy in diabetics can be scored in terms of the blood sugar level achieved one hour after a meal as

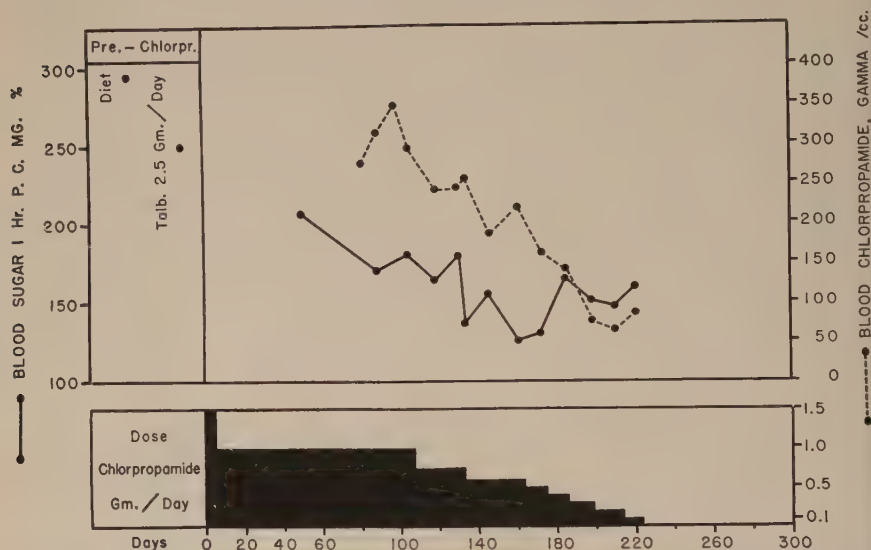


FIGURE 4. Diabetic patient J. S.: a long-term study; uniformly good therapeutic effect over a wide range of successively decreasing dosage.

follows: fair or partial regulation, 190 to 250 mg. per cent (Folin-Wu); good, 150 to 190 mg. per cent; and excellent, below that level.

Even in the less responsive patients, with an end result of only partial regulation, the maximum effective dose is still no greater than 0.5 to 0.6 gm./day, with a corresponding average blood chlorpropamide level of 150 gamma per cc. Raising the dose to 1.5 gm./day with blood chlorpropamide levels up to the range of 275 to 325 gamma/cc. causes no greater lowering of blood sugar level than a dose of 0.5 to 0.6 gm./day. The maximum effective dose can be demonstrated better in those with good regulation. Hypoglycemia never occurred even at the higher levels, although symptoms of overdosage were produced, as discussed below.

In the more responsive diabetics, increase in dosage over a range of 50 mg. up to 0.6 gm./day may or may not improve regulation from good to excellent. When such improvement did occur, it frequently could be correlated with a detectable level of dose and blood level of chlorpropamide. The reverse

could be demonstrated on the reduction of dosage level from the effective levels.

In some of the most responsive patients, not necessarily the mildest in terms of insulin needs, the excellent blood sugar response continued even at the low dosage level of 50 mg./day. The latter was associated with blood chlorpropamide levels only slightly above that of the blank of the method. In several such cases the drug was omitted, and the resultant rise in blood sugar affirmed the activity of such small doses.

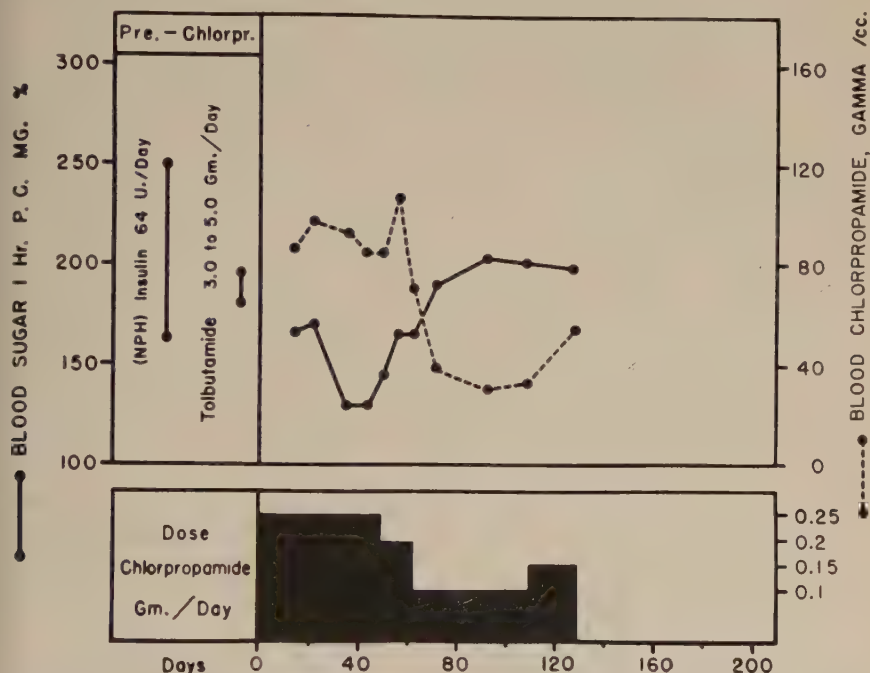


FIGURE 5. Diabetic patient B. G.: a long-term study; successful replacement of 64 units of insulin and clinical titration of minimum effective dose and blood level of chlorpropamide.

The following cases illustrate these principles.

Case 1: Patient J. S. (FIGURE 4), a 33-year-old white male with diabetes of 7 years' duration. He had only a partial effect on 2.5 gm. of tolbutamide per day (blood sugar, 250 mg. per cent, Folin-Wu, 1 hour after meals). On chlorpropamide he had a definitely better effect. The inordinately large initial dose did not produce hypoglycemia although it did produce lethargy and other symptoms. Decrease in dose and blood levels of chlorpropamide was accompanied by no decrease in effect on blood sugar. Indeed, there is a suggestion of increasing effectiveness with time in the face of decreasing dosage, even down to the dose of 0.1 gm./day.

Case 2: Patient B. G. (FIGURE 5), a 64-year-old white male with diabetes of 5 years' duration regulated by 64 units of NPH and regular insulin. Replacement of insulin by tolbutamide (3.0 to 5.0 gm./day) in March 1954 resulted

in better regulation. Even better regulation was achieved on chlorpropamide (0.25 gm./day). Titration by very gradual reduction in dose showed a definite rise in blood sugar at a dosage level of 0.1 gm./day at a blood chlorpropamide level below 70 gamma/cc. The effective replacement of so large a dose of insulin by so small a dose of sulfonylurea is striking.

Case 3: Patient F. B. (FIGURE 6), a 79-year-old female diabetic with diabetes discovered April 1958, with an initial blood sugar of 350 mg. per cent. Tolbutamide (2.0 gm./day) produced an excellent therapeutic response.

EFFECT OF CHLORPROPAMIDE THERAPY ON BLOOD SUGAR AND CHLORPROPAMIDE LEVELS (F. B.)

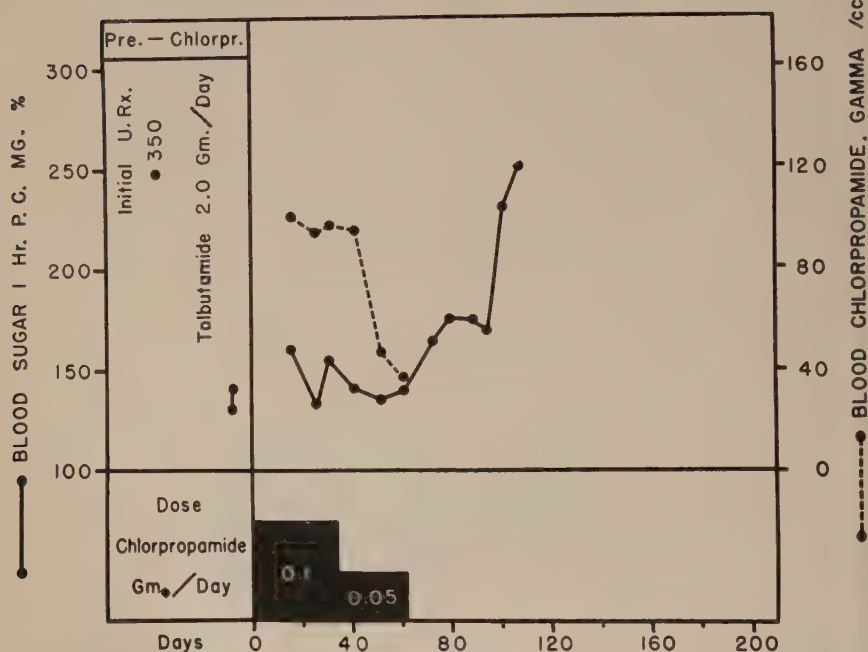


FIGURE 6. Diabetic patient F. B.: a long-term study; low dosage and blood level requirement of chlorpropamide in mild diabetes; proof of effectiveness by withdrawal of drug.

Chlorpropamide therapy was begun in May 1958 with an initial dose of 0.1 gm./day. The response was maintained on reduction of dose to 50 mg./day and a blood chlorpropamide level of 28 gamma/cc. Omission of all therapy resulted in a gradual rise in blood sugar to 250 mg. per cent over the next 40 days.

The dose dependency of chlorpropamide noted in patients studied with total replacement of insulin could also be ascertained by titration in severe diabetics in whom insulin could not be entirely omitted. In one such instance chlorpropamide (0.4 gm./day) replaced 5 units of insulin at a blood level of 159 gamma/cc., while 0.6 gm./day replaced 11 units of insulin at a blood level of 210 gamma/cc.

Of the 60 patients studied, several were given excessively large doses deliberately or because of the exploratory nature of the early therapeutic studies. Nine patients had definite symptoms of overdosage, consisting of subjective muscular weakness, a "groggy or fuzzy" feeling, lassitude, numbness and tingling in toes, and nausea. All managed to perform their mental tasks, although with an overlay of lassitude and sleepiness. Ataxia occurred in one exceptional individual who received 3.0 gm./day.

The daily dosage levels associated with such symptoms were: 1 patient, 3 gm.; 2 patients, 1.5 gm.; 3 patients, 1.0 gm.; 1 patient, 0.75 gm.; and 1 patient, 0.4 gm. There was a definite correlation with blood chlorpropamide level, which was usually over 250 to 275 gamma/cc. at the time of symptoms. This was underscored by the exceptional patient who had symptoms at a dosage level of 0.4 gm./day while, due to unusually slow disposal, the blood level was 280 gamma/cc. However, other patients were asymptomatic at blood levels of 275 to 295 gamma/cc., as were others on doses of 0.75 to 1.0 gm./day. This illustrates certain individual factors involved in the subjective symptoms of overdosage.

All symptoms of overdosage disappeared readily within 4 to 5 days or less of reduction in dosage even in the most highly overdosed individual (3.0 gm./day), with no residual symptoms.

Finally, the average and usual dose of definitive regulation of all these cases was 0.1 to 0.4 gm./day, so that the 0.75- to 1.5-gm. dose productive of symptoms was 2 to 5 times the therapeutically necessary dose.

Discussion

It is of considerable theoretical importance that there is a proportionality between oral dosage and blood levels (and, presumably, hypoglycemic activity) of chlorpropamide in treated diabetics; this is especially true in individual patients. The relationship is closest with least variability in blood chlorpropamide levels in individual patients on a given dose of chlorpropamide, who may show remarkably constant blood levels for several weeks at a time. No accumulation of drug in the blood occurs even after months of therapy.

Since patients vary widely in the dose required for optimal regulation, this leads to the distinct clinical advantage that the drug may be administered in varying doses without determination of blood levels. However, individual patients do show variations from one another in their blood response. This helps to explain unexpected treatment failures and suggests the judicious elevation of the dose of chlorpropamide above the usual maximum (0.6 gm./day) in such patients.

The range of effective blood levels is quite wide: from 25 to 175 gamma/cc. The effectiveness at the low dosage and blood levels in responsive patients is striking.

While clinical experience suggests that the other sulfonylureas (carbutamide and tolbutamide) probably also have the same dose-blood sulfonylurea response relationship, this type of extensive long-term study has not been performed for those drugs.

The risk of hypoglycemia is small in spite of the great clinical effectiveness of chlorpropamide. The level of overdosage is far beyond the usual therapeutic range, and its symptoms are so mild and easily recognizable as to present no clinical problems. It is of interest that one symptom, muscular weakness of the lower extremities, has been produced in the experimental animal by very large intravenous doses of tolbutamide (270 mg./kg./hour), and is due to direct action on the spinal cord.²

Summary

Chlorpropamide has been administered to 60 diabetics over a period of 8 months. Both clinical response and blood chlorpropamide levels were measured. In general, there is a close relationship between dose and blood chlorpropamide response, but there are some individual variations among patients in such response. Patients maintained blood chlorpropamide levels within narrow limits at a given dosage level over long periods of time. There is no accumulation of the drug after long periods of therapy. The routine determination of blood chlorpropamide is unnecessary in clinical regulation of diabetics, although it may be useful in some nonresponsive patients.

The effective dosage range in the treatment of diabetics is 50 mg. to 0.6 gm./day, and the corresponding blood levels are 25 to 175 gamma/cc. In some patients a demonstrable level of blood chlorpropamide for effectiveness may be determined. Hypoglycemia is rare, and symptoms of overdosage are mainly mild weakness. This does not usually occur below a dose of 0.75 to 1.0 gm./day, which is beyond the usual therapeutic range.

Acknowledgment

The statistical analysis of the data used in the preparation of FIGURE 1 was performed by Hilda Simon of the Statistical Department of Chas. Pfizer & Co., Inc.

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CLINICAL EXPERIENCES WITH CHLORPROPAMIDE, WITH SPECIAL REFERENCE TO COMPARISON WITH PREVIOUS EXPERIENCE WITH CARBUTAMIDE AND TOLBUTAMIDE

H. A. Heinsen

Medical Clinic and Diabetic Department, Waldkrankenhaus, Zeven, Germany

At this writing it is about 3 years since we first used sulfonylurea preparations in Germany for the treatment of diabetics. During this period we have employed these drugs systematically and on a large scale. A short time ago I reported our accumulated experience with these new therapeutic agents. In that summary I emphasized the fact that there can no longer be any doubt that oral sulfonylurea therapy has proved to be of value in the treatment of diabetes despite the fact that the mechanism of action of these substances remains to be clarified completely. Approximately 40 per cent of all diabetics can be treated successfully by means of oral administration of these antidiabetic drugs, and the metabolism can be stabilized satisfactorily for the duration of the therapy. The number of "late failures" did not exceed 10 per cent. The incidence of undesirable side reactions was 8 per cent in the case of carbutamide (BZ 55) and less than 1 per cent in the case of tolbutamide (D 860).

Carbutamide and tolbutamide are the 2 substances that heretofore have been generally available in Germany for oral sulfonylurea therapy. Preference has been given to the latter, both in Germany and, to the best of my knowledge, in the United States also, because it does not possess sulfonamide characteristics. Therefore it is significantly better tolerated and exhibits an incidence of untoward side reactions considerably smaller than that of carbutamide.

Recently, experimental and clinical studies have been conducted in the United States on chlorpropamide, a new sulfonylurea derivative. We have been the first investigators to use this new antidiabetic drug in Germany, employing it for the treatment of diabetics in the Diabetic Pavilion of the Waldkrankenhaus. We had available information supplied by the manufacturer concerning the physical and chemical properties of chlorpropamide, its intensive hypoglycemic action in the dog and rat, and the observed lack of damage to the internal organs of these test animals.

Materials and Methods

Altogether, we have completed control with chlorpropamide in 50 hospitalized diabetics. In an additional 24 cases, full metabolism adjustment has not yet been completed. While this is an admittedly rather small test group, the patients were very carefully examined and specific tests were conducted to determine the effect of therapy on carbohydrate metabolism and to detect any untoward effect which might develop. All patients received thorough examinations at intervals of several weeks, beginning with

the initiation of treatment. I therefore believe that we can offer certain valid conclusions on the basis of our experiences.

TABLE 1 presents the composition of our test group according to age. As may be seen, the 50- to 60-year age group clearly predominates with 23 patients. Eleven other patients were between 40 and 50 years of age. In Germany we classify adult diabetics (those having maturity-onset diabetes) chiefly into 2 categories: first, the sthenic type, which includes patients who

TABLE 1
AGE GROUPS OF THE DIABETICS TREATED WITH CHLORPROPAMIDE

Age (years)	Men	Women	Total
21-30	2	1	3
31-40	—	—	—
41-50	4	7	11
51-60	12	11	23
61-70	1	6	7
71-80	1	5	6
	20	30	50

TABLE 2
DURATION OF THE DIABETES BEFORE INITIATION OF CHLORPROPAMIDE THERAPY

	Newly diagnosed	Time (years)								
		1/2	1	1 1/2	2	3	4	5	7	More than 7
Number of patients.....	10	3	4	3	7	6	6	6	1	4

appear well nourished and in whom insulin output may be considered to be normal or near normal, but who develop the disease late in life due to the effects of other endocrine glands on the metabolism of the patient; and, second, the asthenic type, which includes patients who are thin, not well nourished, who suffer easily from fatigue, and whose insulin output may be considered to be deficient.

It is the sthenic type of diabetic who generally responds to therapy with oral antidiabetic agents; the asthenic type usually is not amenable to such therapy. The majority of our patients were of the sthenic type, and only 2 could be considered as definitely of the asthenic type.

TABLE 2 indicates the duration of diabetes in each individual case; as shown, the minimum duration is six months, the maximum more than seven years. It would appear, however, that the duration of the disease is not an important criterion for determining the advisability of initiating sulfonylurea

asthenic type in whom diabetes is a result of a pronounced lack of insulin, chlorpropamide seems to be just as unsuitable as the other two sulfonylurea preparations. Of course, there are exceptions to this general rule; indeed, the treatment of all diabetics must be so carefully based on each individual case that one can say there is hardly any other disease that requires such strict individual attention. Aside from this, we had the impression in a few other cases that the use of chlorpropamide was possible in cases where carbutamide and tolbutamide had previously failed to bring about metabolic compensation at the same dosage levels. Two cases illustrating this point are shown in

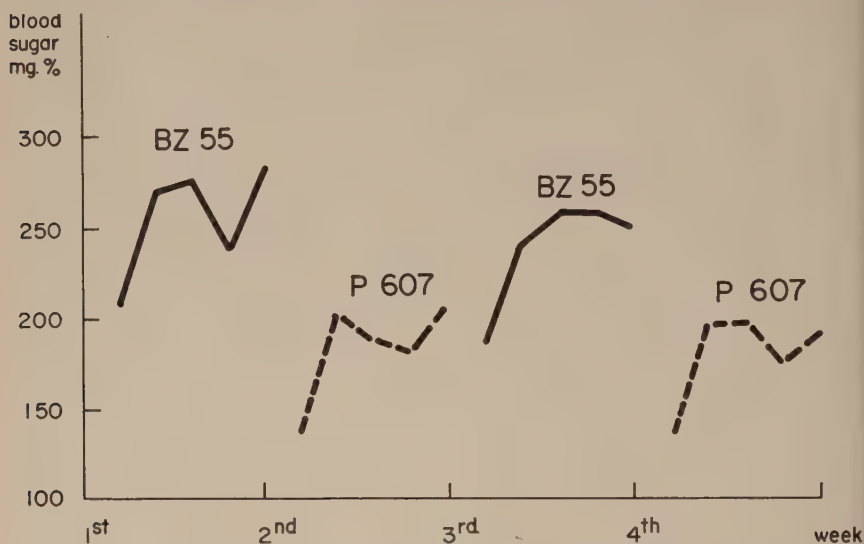


FIGURE 1. Blood sugar levels in relation to treatment with chlorpropamide and carbutamide. Patient F. W. H., male, 53 years old. Dosage was 1 tablet twice daily (1000 mg. daily).

FIGURES 1 and 2. In FIGURE 1, one carbutamide tablet twice daily, for example, was insufficient to maintain the metabolism in balance; the daily blood-sugar profile showed values as high as 280 mg. per cent. In contrast, 1 chlorpropamide tablet twice daily maintained blood sugar values at considerably lower levels, never exceeding 200 mg. per cent. FIGURE 2 shows a similar situation in regard to tolbutamide as compared with chlorpropamide therapy.

I should also like to call your attention to 2 other points. First, when equivalent dosage of the antidiabetic agents is administered, the hypoglycemic action of chlorpropamide is definitely more potent than that of tolbutamide. Second, the fasting blood sugar, especially, is lower when chlorpropamide is administered. We observed these effects quite frequently in our test patients, and thereby we were able to come to important conclusions regarding dosage for chlorpropamide.

At the beginning of our tests we generally used an initial dosage of 500 mg. of chlorpropamide three times daily. This dosage was never exceeded, and

we continued to administer it until a definite improvement in metabolic compensation was evident, with urine sugar and blood sugar values approaching normal. As our experience accumulated, we learned that for a number of patients an initial dose of 500 mg. twice daily would achieve the desired result and, in a few cases, we were able to initiate therapy with a single daily dose of 500 mg. of chlorpropamide. This was possible, however, in only a small proportion of the total cases. Furthermore, the initial dosage, as well as subsequent maintenance dosage, required adjustment to individual need,

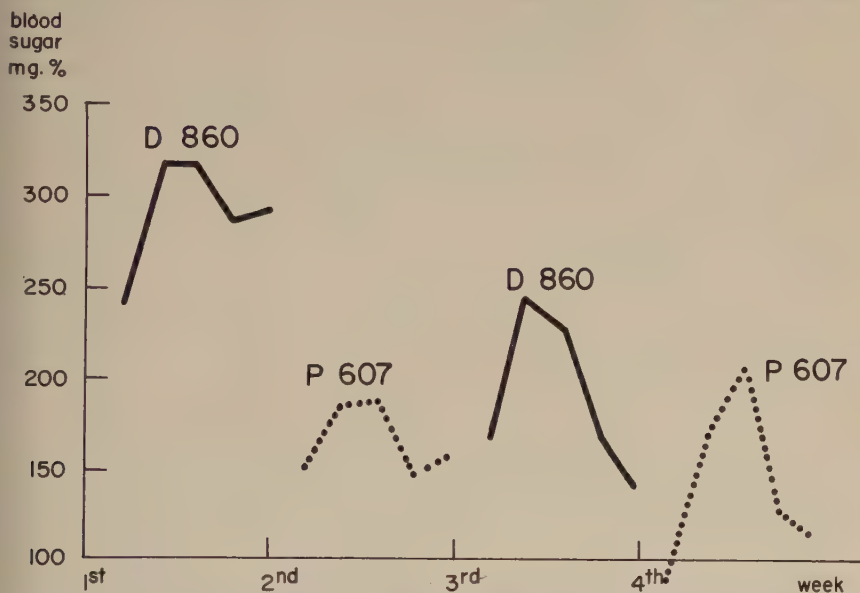


FIGURE 2. Blood sugar levels in relation to treatment with chlorpropamide and tolbutamide. Patient M. W., female, 57 years old. Dosage was 1 tablet twice daily (1000 mg. daily).

to the individual response of each patient, to the tempo at which the compensation of the metabolism progressed, and to other such factors. Thus, as with all antidiabetic medication, over-all indications can be given for dosage, but no hard and fast precise directions can apply to all individuals. In a number of our patients we observed that satisfactory metabolic compensation could be obtained only if the initial dosage was 500 mg. three times a day, administered for several days.

With occurrence of satisfactory metabolic compensation the initial dosage was gradually reduced after 4 to 6 days, being replaced by a maintenance dosage of 125 to 1000 mg./day, as shown in TABLE 5. Few patients required a daily maintenance dosage as high as 1000 mg., and these were mostly cases whose sugar metabolism had not been maintained in balance by the same dosage of carbutamide or tolbutamide. In some of these cases examination a few weeks later revealed that metabolic compensation had improved to such an extent that the daily maintenance dosage could be reduced to 500 mg.

or, in some cases, even to 250 mg. According to my recollection, this is an observation we scarcely ever encountered during our earlier investigation of tolbutamide. With some reservations, one can perhaps conclude from this observation that under chlorpropamide medication the poorly functioning pancreas improves somewhat and again begins to produce insulin in more adequate amounts.

From the facts I have already presented, it is clear that it is still somewhat premature to make any definitive statement concerning the maximal initial and maintenance dosages of chlorpropamide. As I have stressed, this must be a matter of individual adjustment. Nevertheless, we do have the impression that the maintenance dosage is frequently considerably lower than with

TABLE 5
MAINTENANCE DOSE OF CHLORPROPAMIDE

	Chlorpropamide (mg.)					
	1000	500	250	250 every second day	125	125 every second day
Number of patients.....	9	14	13	1	6	1

carbutamide and tolbutamide, so that in many cases the sugar metabolism can be balanced satisfactorily as long as chlorpropamide maintenance dosages on the order of 125, 250, or 500 mg. are continued. Only conjectures can be offered for explanation of this behavior. Chlorpropamide is absorbed very quickly from the gastrointestinal tract. On the other hand, the substance is eliminated from the body very slowly. Because of these factors it is possible to preserve a blood level of chlorpropamide high enough to accomplish a satisfactory hypoglycemic action with very small daily dosage. It is also possible that the explanation of the more potent hypoglycemic effect of chlorpropamide lies in the same factors. All of these questions require further investigation. As I have already indicated, we were impressed throughout our study by the fact that the fasting blood sugar frequently was quite low. For this reason we have preferred to administer the drug early in the day and according to patient need, giving either a daily dosage of one-half or one full tablet immediately after breakfast as a single dose or, where daily dosage is required, of 1 tablet twice daily; that is, after breakfast and after lunch.

Patients previously treated with insulin can be successfully placed on chlorpropamide therapy, just as is the case with carbutamide and tolbutamide. With such patients we were able to replace daily insulin dosage as high as 48 units with chlorpropamide. One of these cases is shown in FIGURE 3. Heretofore the patient had taken 32 units of insulin daily. Five hundred mg. of chlorpropamide daily did not produce a complete normalization of the blood sugar values, which we consider the necessary criterion for the lasting

success of oral diabetic treatment. With 2 daily doses of 500 mg. each, the blood sugar was completely normalized, so that the use of insulin could be discontinued. It is worthy of mention that, in a number of newly diagnosed diabetics who suffered from acidosis before initiation of treatment, we did not use insulin at all, but tested the effectiveness of chlorpropamide by administering it from the beginning as the only antidiabetic therapy given. The acidosis disappeared and the metabolism was normalized. This was a most interesting observation, but it was merely in the nature of a clinical

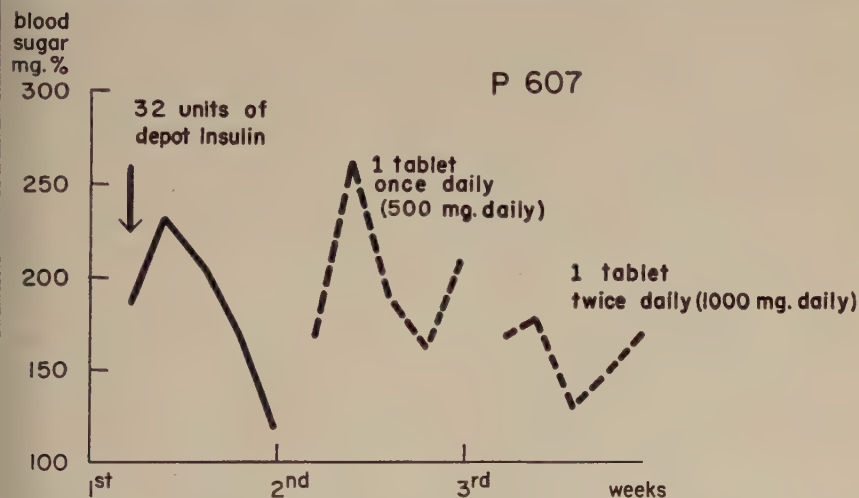


FIGURE 3. Blood sugar levels in relation to treatment with chlorpropamide and insulin. Patient W. S., male, 52 years old. Dosage was as shown.

experiment, and I do not recommend it as a regular procedure to be followed by other investigators.

Only twice did we encounter so-called late failures. These were patients who responded well to chlorpropamide treatment and who were discharged on a maintenance dosage of 500 mg. twice daily. After 2 to 3 months they returned for examination, and it was found that in both cases the metabolism was decompensated. Even when the dosage was increased to 500 mg. three times daily there was no improvement, so that it was necessary to reinstitute insulin therapy.

Side Effects

We devoted particular attention to the question of whether chlorpropamide had any deleterious side reactions such as may be seen to some degree with carbutamide. The following may be reported: up to a dosage of 500 mg. three times a day we observed no episodes of shock. We could detect no damage to the liver, the kidneys, or the hematopoietic system. Significantly, the continued liver function tests and blood examinations invariably revealed normal results. In regard to possible toxic effects, one case is particularly interesting. A 63-year-old female diabetic came to us with severe jaundice following an attack of hepatitis. Serum bilirubin amounted to 15 mg. per

cent, and tests of liver function gave abnormal results, as expected. Heretofore the patient had been taking 40 units of insulin daily, which we replaced with chlorpropamide. A maintenance dosage of 500 mg. daily was established. The jaundice disappeared while the patient was under treatment, but 6 weeks after discharge hepatic function tests still gave abnormal values. After 2 months, carbohydrate tolerance had improved so much that the daily chlorpropamide dose could be reduced to 250 mg. After an additional month and one half of therapy, examination of the liver showed completely normal results: the liver was no longer enlarged, and the function tests gave normal results. The patient felt healthy and had gained weight. This case shows not only that hepatic damage need not be feared with chlorpropamide, but also that existing hepatic damage can heal during chlorpropamide treatment.

The appearance of a pruritic measleslike exanthema was noted in three patients some four to six weeks after initiation of medication with chlorpropamide therapy. This side reaction disappeared a few days after the drug was discontinued. Examination of metabolism indicated that further administration of chlorpropamide was no longer necessary, since metabolic balance could now be maintained by dietary means alone. A so-called thrust effect of the sulfonylurea substances occurred; we had seen this previously when using carbutamide and tolbutamide. Furthermore, in these cases there did not appear to be a drug allergy to chlorpropamide, since the intracutaneous tests and the Prausnitz-Küstner test were negative.

Using an initial dosage of 500 mg. three times daily, we observed a number of cases of gastrointestinal disturbances, principally epigastric distress, feelings of pressure in the stomach, and nausea. For the most part, however, these disappeared when the dosage was decreased, so that maintenance dosages of 250 to 500 mg./day were only rarely accompanied by epigastric manifestations. Interesting in this connection is the reaction of a few patients who had also complained of epigastric distress when carbutamide and tolbutamide were used. When these patients received the smaller, but fully effective, dosage of chlorpropamide, they no longer complained of epigastric distress. Apparently they tolerated a therapeutic amount of chlorpropamide better than they did either of the other 2 sulfonylurea preparations.

Conclusion

Again I stress most emphatically the fact that our clinical investigation of chlorpropamide is preliminary in nature and involves tentative tests conducted with a relatively small group of test patients; hence, it is not feasible to come to final and definitive conclusions from this work. Further research should be conducted to establish validity in regard to indications, tolerance, side reactions, and toxicity. In future investigations it will be important to explore further possible side effects that may involve the hepatic or hematopoietic systems. As I have indicated, we have never seen any sign of such effects, but other investigators have reported occasional occurrences. Therefore, further evaluation of this new oral antidiabetic agent is necessary.

CLINICAL EXPERIENCES WITH CHLORPROPAMIDE GIVEN TO HOSPITALIZED PATIENTS

Garfield G. Duncan

*University of Pennsylvania, Pennsylvania Hospital, and the Benjamin Franklin Clinic,
Philadelphia, Pa.*

Guy L. Schless, Mohamed M. Ali Demeshkieh*

Pennsylvania Hospital, Philadelphia, Pa.

Introduction

Oral therapy for diabetes is no longer a novelty. As months have grown into years of experience with oral sulfonylurea compounds, the apprehension that harm might ensue from their use over long periods is subsiding. As a result, search for the most effective compound with the least toxicity has been intensified. This paper deals with a clinical testing of chlorpropamide in 15 hospitalized diabetic patients with a follow-up of 6 months. The duration of the diabetes ranged from 1 week to 16 years (TABLE 1). Seven patients received a daily average dose of 30 U. of insulin prior to the test period. One was taking 55 U. daily. Two patients were receiving tolbutamide with unsatisfactory results. In 6 patients the diabetes was not under good control; none of these was receiving insulin or tolbutamide.

Ideally, the patients for chlorpropamide therapy would be selected from approximately 10 per cent of the diabetic population. This is based on the relatively sound assumptions that it is possible to control diabetes, barring acute complications, in 80 per cent by diet alone, that 5 per cent are children, and that the remaining 5 per cent are adults with labile diabetes. In these two latter groups long-term treatment with chlorpropamide is ineffective.

Method

A few preliminary days of observation preceded the test period that consisted of a minimum of 3 consecutive 5-day periods. During the first 5 days the amount of insulin given was reduced or eliminated and, in the case of patients receiving tolbutamide, this drug was withdrawn. In each instance the object was to permit hyperglycemia and glycosuria to develop in order to discern the effect of chlorpropamide more clearly. During the second 5-day period chlorpropamide was administered and, throughout the third period, this drug was omitted. Throughout the 15 days, daily blood sugar determinations were made by the Somogyi-Nelson method on samples taken in the fasting state and, at 3 P.M., with daily quantitative determinations of the glycosuria. Tests for acetonuria were performed on successive voidings when the insulin therapy was reduced. Other laboratory studies were made during the first and toward the end of the second week. The diet remained constant throughout and patients were guarded against undernutrition.

* Research Fellows in Metabolism.

TABLE 1
DATA ON CHLORPROPAMIDE THERAPY AND RESULTS IN 10 CASES OF FAVORABLE RESPONSES

Case No.	Patient age color sex	Duration of diabetes	Prior treatment	Percentage fall		24-Hour quantity in urine	Chlorpropamide daily (gm.)	Other
				Fasting	3 P.M.			
1	MG 66WF	1 month	Insulin 10 U.	56.3	41.5	—	0.5	
2	JR 42WM	7 years	Diet	45.5	33.7	69.7	0.5	Tolbutamide failure
3	AM 79WM	1 week	Insulin 8 U.	47.1	51.2	37.5	0.5	
4	CS 60WF	2 weeks	Insulin 15 U.	74.1	58.2	92.3	0.25	Chlorpropamide 0.5 gm. daily
5	CR 73WF	6 months	Tolbutamide 1.5 gm.	52.6	14.8	71.4	1.0	Tolbutamide failure
6	GR 76CM	6 months	Tolbutamide 1.5 gm.	57.1	40.1	88.5	1.0	Tolbutamide fair control
7	BS 57WF	16 years	Insulin 40 U.	60.5	54.9	91.5	1.0	
8	LB 68WM	16 years	Insulin 36 U.	33.0	27.4	98.8	0.75	Tolbutamide failure
9	EJ 85WF	6 years	Insulin 44 U.	44.1	24.4	—	0.75	
10	JG 51CF	2 weeks	Insulin 55 U.	4.2	11.6	46.2	1.0	
Range		1 week to 16 years	Insulin 8 to 55 U.	4.2 to 74.1	11.6 to 58.2	37.5 to 98.8	0.25 to 1.0	
Average		4.6 years	Insulin 30 U.	47.5	35.8	87.0	0.73	

Results

In 10 cases no acetonuria was detected during the first 5-day control period and in each instance chlorpropamide effectively controlled the glycosuria and hyperglycemia during the second 5-day test period. In 5 cases either acetonuria occurred promptly (FIGURE 1) or acute complications existed. In none of these was adequate control of the diabetes achieved with chlorpropamide, and resumption of insulin therapy was necessary. One patient who, prior to the test, had been receiving 1.5 gm. of tolbutamide daily, had fasting blood sugars ranging from 271 to 342 mg. per cent with an average of 296 mg. per cent. Chlorpropamide resulted in a prompt reduction of the fasting blood sugar to 82 mg. per cent. The comparative effects of tolbutamide and chlorpropamide, as observed during the period of hospitalization, indicated (TABLE 2) that chlorpropamide in this instance is a more potent blood sugar reducing agent than tolbutamide. This impression was supported by at

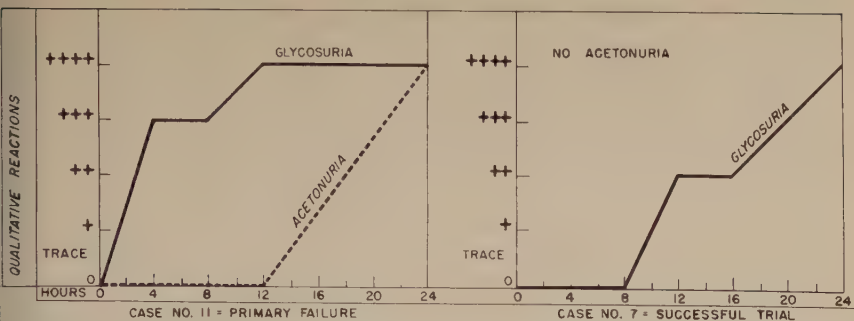


FIGURE 1. Patients who promptly developed acetonuria upon reduction or withdrawal of insulin, illustrated in case 11, did not respond favorably to chlorpropamide. However, the failure of ketonuria to appear, as in case 7, is a rather reliable indication that chlorpropamide will exert a favorable effect.

Follow-up of 6 months' duration, during which time 1 gm. of chlorpropamide was given daily; the fasting blood sugar values ranged from 147 to 210 mg. per cent, with an average of 194 mg. per cent. The other patient, who had been taking 1.5 gm. tolbutamide daily for 6 months, had fasting blood sugar values ranging from 161 to 232 mg. per cent, with an average of 184 mg. per cent. A prompt decrease in the fasting blood sugar to 84 mg. per cent followed the use of chlorpropamide, one dose of 1.5 gm. daily, and during the ensuing 3 months with a dosage of 0.5 gm. of chlorpropamide; the fasting blood sugar values were normal, varying from 105 to 127 mg. per cent.

In 2 cases in which tolbutamide had been a recent secondary failure, excellent control of the diabetes was achieved with chlorpropamide. The drug had a blood sugar lowering effect in 10 of the 15 patients.

Following discharge from the hospital of these 10 patients the diabetes in each of them has been controlled by 0.25 to 1.0 gm. of chlorpropamide daily. The average amount given in 1 dose daily was 0.75 gm. Experience with this drug and hypoglycemic reactions caused by it have prompted us to begin therapy with a daily dose of 0.5 gm. and modify it as needed. Addi-

TABLE 2
EFFECTS OF IDENTICAL DOSES OF CHLORPROPAMIDE AND TOLBUTAMIDE ON THE BLOOD SUGAR VALUES AND ON THE DEGREE OF GLYCOSURIA IN ONE PATIENT

	Average			6 Month follow-up average & range blood sugars, mg. per cent
	FBS mg. per cent	3 P.M. Blood sugar—mg. per cent	Urine sugar gm./24 hour	
retest control.....	228	284	9.8	194 (147-210) 296 (271-342)
chlorpropamide, 1.0 gm....	147	168	2.1	
tolbutamide, 1.0 gm.....	184	262	11.3	

tions of more than 0.25 gm. to the daily dose in 1 week increased the risk of hypoglycemic reactions.

A typical favorable response to chlorpropamide therapy is illustrated in FIGURE 2. Both fasting and postprandial hyperglycemic values were corrected. Furthermore, following withdrawal of the drug a gradual increase of the blood sugar to abnormally high levels ensued.

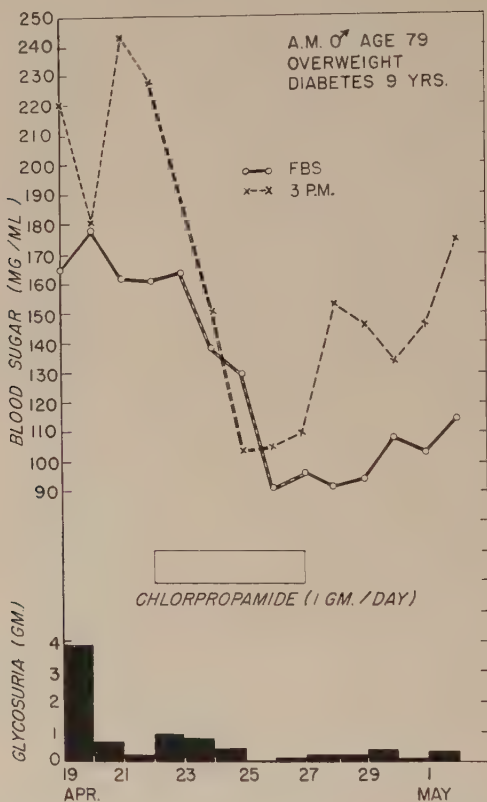


FIGURE 2. The hyperglycemia and glycosuria observed in the preliminary control period were corrected by 1.0 gm. of chlorpropamide daily. The favorable effect gradually subsided after the chlorpropamide was omitted.

Primary Failures

Five primary failures with chlorpropamide therapy were noted. In 2 cases acetoneuria appeared in increasing amounts in the first 24 hours after the withdrawal of insulin, as illustrated in FIGURE 1. In another case an acute cholecystitis with cholelithiasis was a complicating factor; in 1 case metastatic carcinoma was present; there was 1 patient who had gangrene of an extremity.

Data in the case of a failure to respond favorably to chlorpropamide are

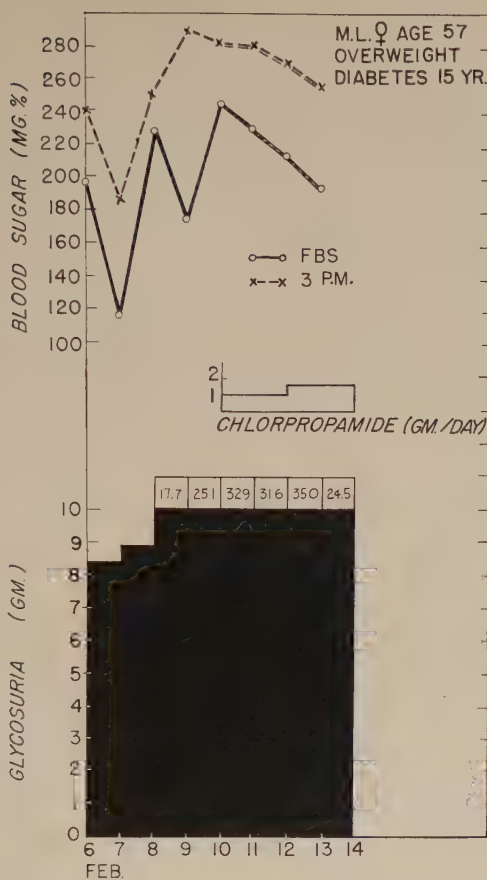


FIGURE 3. The hyperglycemia and glycosuria were not significantly improved in this case during five days of chlorpropamide therapy.

shown in FIGURE 3. Neither the hyperglycemia nor the degree of glycosuria was favorably influenced by the drug.

Side Reactions

Hypoglycemic reactions, mild in nature, were accompanied in two cases by nervousness, excessive perspiration, irritability, and sleepiness. In each of these patients an abrupt reduction of the level of the blood sugar followed the administration of 1 gm. of chlorpropamide. A reduction of the dose by 0.25 gm. prevented hypoglycemic reactions.

A transient pruritic, maculopapular cutaneous eruption occurred on 1 forearm of 1 patient who had received 1 gm. daily of the drug for 1 month. The rash receded under influence of antihistamine therapy and there has been no recurrence without interruption of chlorpropamide therapy.

Special Tests

An abnormal alkaline phosphatase value was noted in one instance, but this was doubtless due to right-sided heart failure with auricular fibrillation. This patient died 1 month after these studies were completed. The liver exhibited a marked central vein congestion considered characteristic of the cardiac disease, with no other detectable abnormalities. In none of the remaining 14 patients was there any detectable disturbance in the hematopoietic, renal, or hepatic functions.

Acute Effect of a Single Dose of Chlorpropamide

The speed of action, degree of effect, and the duration of the blood sugar lowering effect of a single dose of chlorpropamide were observed when a liquid dietary formula comprised of protein, 113 gm., carbohydrate, 268 gm., and fat, 63 gm. (2090 calories) was divided into 12 equal amounts, 1 of which was

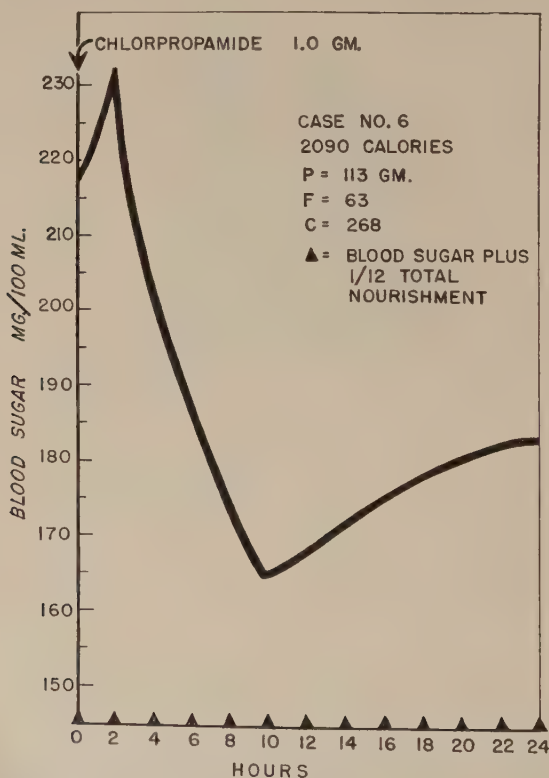


FIGURE 4. The effect of a single dose (1 gm.) of chlorpropamide is illustrated. Identical liquid nourishments were given at 2-hour intervals for 24 hours. The blood sugar lowering effect became apparent between 2 and 4 hours after the first nourishment was given. This effect was most marked between 8 and 12 hours, but was still apparent after 24 hours had elapsed.

given every 2 hours throughout the 24 hours. As illustrated in FIGURE 4, no hypoglycemic effect from 1 gm. of chlorpropamide was apparent in the first 2 hours, but an appreciable decrease in the level of the blood sugar had developed within 4 hours, with a maximum blood sugar lowering effect at 10 hours, after which a slow and very gradual increase began. A marked depressing effect on the level of the blood sugar was still manifest 24 hours after the drug had been administered.

The effect of 0.5 gm. given twice daily is compared in TABLE 3 with that of 1 gm. given in 1 dose. The single dose produced the more consistent

TABLE 3
DEGREE AND DURATION OF EFFECT OF 0.5 GM. OF CHLORPROPAMIDE ON THE FASTING AND POSTPRANDIAL BLOOD SUGAR LEVELS AS COMPARED WITH THOSE FOLLOWING THE ADMINISTRATION OF 1.0 GM. GIVEN ONCE DAILY

Chlorpropamide	Average		Urine sugar gm./24 hour
	FBS mg. per cent	3 P.M. Blood sugar mg. per cent	
0.5 gm., b.i.d.....	108	242	2.8
1.0 gm., single dose.....	147	168	2.1

blood sugar lowering effect. On this basis, because of similar observations, we give a single dose of chlorpropamide before breakfast daily.

It was observed that with the daily administration of chlorpropamide its apparent effect increased for several days, after which a plateau of effect ensued. Also, after withdrawal of the drug the blood sugar lowering effect abated gradually over a 3- to 5-day period.

No instance of decreasing effect of chlorpropamide during therapy up to and in excess of six months has been observed. It is possible, and indeed probable, that the lack of secondary failures is due to the brevity of the period of therapy.

Summary

This study of the properties of chlorpropamide involving 15 closely observed hospitalized patients indicates that: this drug is a potent blood sugar reducing agent when given in doses as small as 0.25 to 0.5 gm./day; the blood sugar lowering effect becomes apparent between 2 and 4 hours after 1 gm. is administered by mouth and extends beyond 24 hours; after 5 days of therapy the hypoglycemic effect disappears gradually over a period of 3 to 5 days after withdrawal of the drug; it served, with suitable dietary measures, to control the diabetes in 10 of the 15 patients having "adult-acquired diabetes"; in no instance was it necessary, because of side effects, to discontinue the drug, though one patient had a transitory macular skin eruption; there were no significant hematological, renal, or hepatic disorders attributable to this ther-

apy; and the detection of acetonuria in increasing amounts after a reduction of the amount of insulin given served as a reliable guide in detecting those patients in whom a satisfactory response would not be obtained whereas, in cases in which no acetonuria occurred, there were effective responses to chlorpropamide therapy.

Prolonged therapy under close surveillance is still necessary to prove the harmlessness of this drug. Also, the likelihood must be guarded against that such an effective agent might receive widespread adoption as a substitute for appropriate dietary measures.

OBSERVATIONS ON THE MODE OF ACTION AND CLINICAL EFFECTS OF CHLORPROPAMIDE IN DIABETES MELLITUS

Carroll M. Leevy, Abraham Tornow,* Philip Greenberg,
Myra Zinke†

Hepatic Metabolic Research Unit and the Diabetic Clinic, Department of Medicine, Jersey City Medical Center, Jersey City, N. J., and Seton Hall College of Medicine and Dentistry, Jersey City, N. J.

Preliminary clinical studies of chlorpropamide have demonstrated this drug to be an effective hypoglycemic agent in man. In higher dosage ranges

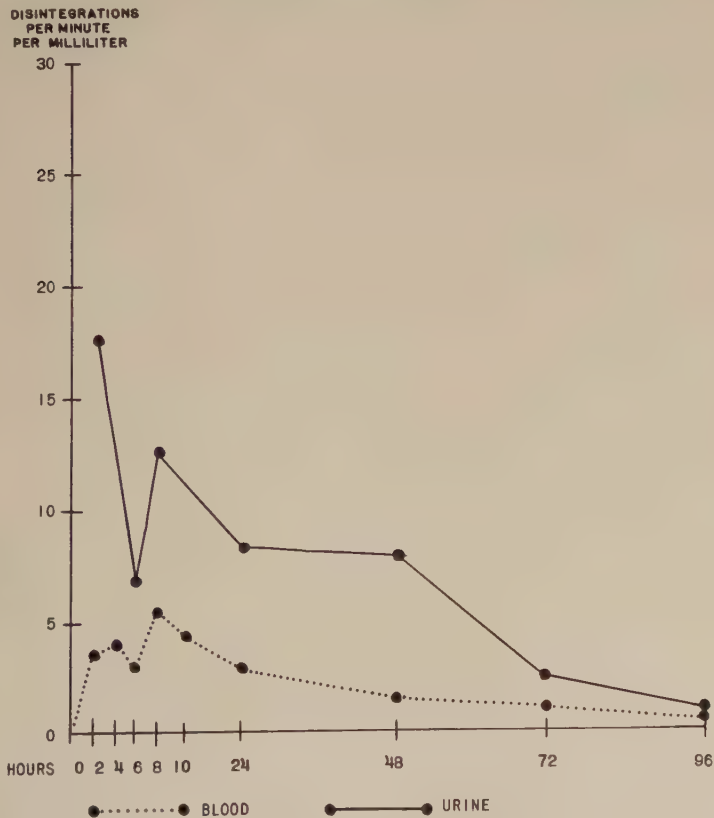


FIGURE 1. Serum and urinary levels after oral administration of 1.0 gm. of S^{35} chlorpropamide.

It has produced marked hypoglycemia in susceptible persons. It has permitted control of clinical and laboratory abnormalities in mild to moderate

* Fellow in metabolism and gastroenterology.

† Trainee, National Institutes of Health, Public Health Service, Bethesda, Md.

diabetes of adult onset, in some instances of brittle diabetes, and in patients who have been refractory to tolbutamide.¹ The potency of chlorpropamide should make it more suitable for studies of the influence of sulfonylurea derivatives on carbohydrate metabolism and, thereby, facilitate investigation of their mode of action in man. This paper records clinical observations on

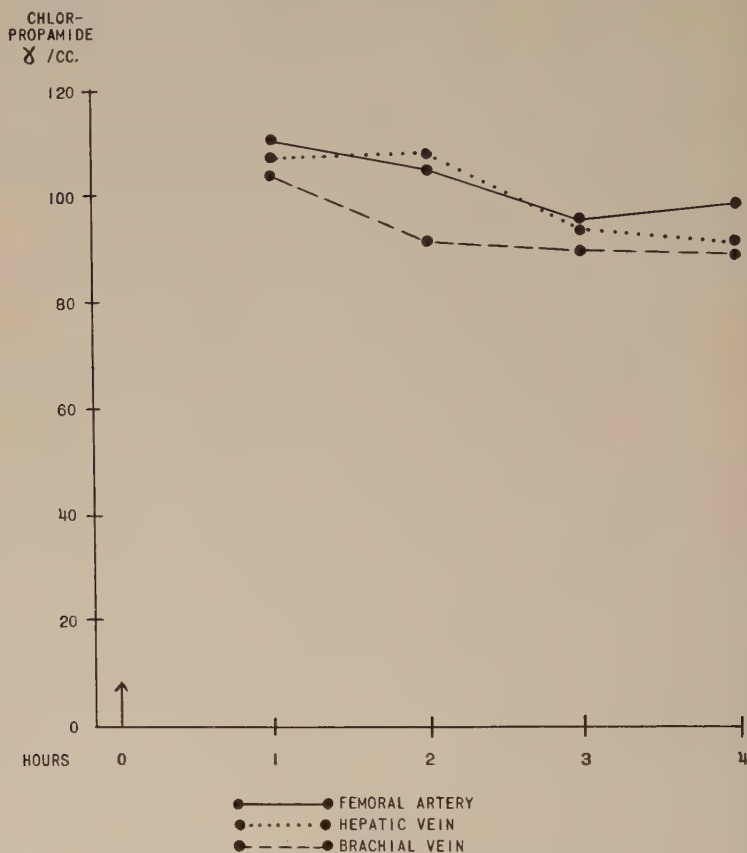


FIGURE 2. Comparison of arterial, hepatic venous, and peripheral venous levels of chlorpropamide after administration of 1.0 gm. of the drug intravenously.

its distribution and excretion; its effects on arterial, venous, and hepatic venous blood sugar levels; its influence on the prednisone glucose-tolerance test and epinephrine-induced hyperglycemia; and experience with it and other sulfonylurea derivatives on diabetes made overt by liver injury.

Materials and Methods

Distribution and excretion were studied in 2 diabetic subjects by measuring urinary output of orally administered S^{35} chlorpropamide and in 10 non-diabetic subjects by determining drug levels in arterial, venous, and hepatic venous blood after oral or intravenous administration of nonradioactive

chlorpropamide. The relationship of drug dosage and serum level to blood sugar changes for a 48-hour period was investigated in 6 patients with diabetes by administering 1.0 gm. of chlorpropamide as a single dose orally. Results of prolonged administration of the drug on its serum concentration were investigated by obtaining weekly determinations in 12 patients receiving 0.5 or 1.0 gm. of chlorpropamide each day. Comparison of arterial, venous, and hepatic vein drug and sugar levels was made in 10 patients by using the technique of hepatic vein catheterization.² Effects of chlorpropamide on

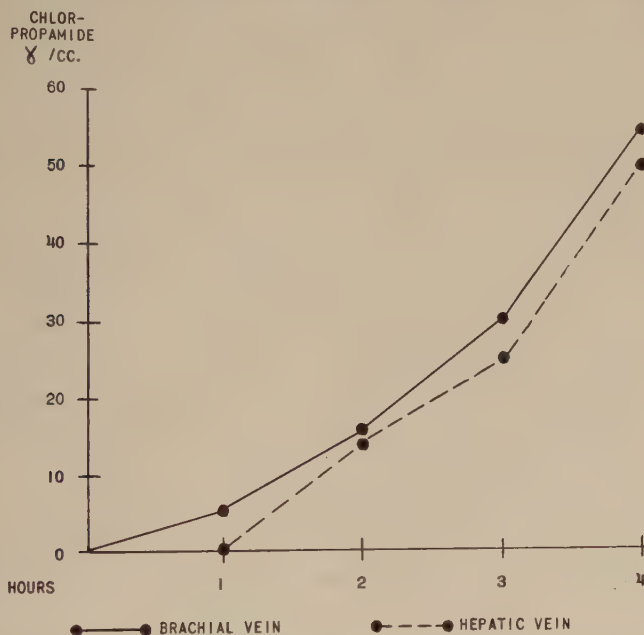


FIGURE 3. Oral absorption of chlorpropamide under condition of hepatic vein catheterization.

glucocorticoid-induced hyperglycemia were studied by performing a standard oral or intravenous glucose-tolerance test, repeating the test with prior administration of cortisone³ or prednisone⁴ and, finally, obtaining a glucose tolerance test after receipt of the adrenal steroid plus 1.0 gm. of chlorpropamide. The influence of chlorpropamide on epinephrine-induced hyperglycemia was evaluated by studying the effects of 0.01 cc. of a 1:1000 solution of intramuscular epinephrine on blood sugar before and after administration of 0.5 gm. of chlorpropamide each day for 5 days. Blood sugars were determined by the method of Folin and Wu. Chlorpropamide was determined in the serum by an ultraviolet spectrophotometric procedure.⁵

Observations

After oral ingestion of 1.0 gm. of S³⁵ chlorpropamide there was a peak rise in serum radioactivity at 4 to 6 hours, followed by a gradual decrease in

serum levels and a concomitant increase in urinary radioactivity in the ensuing 48-hour period. Radioactivity was present in the serum and urine 96 hours later (FIGURE 1). A comparison of drug levels in arterial, venous, and hepatic venous blood after parenteral administration of chlorpropamide showed a small but definite difference due to its uptake and metabolism by the liver and other tissues and excretion by the kidney (FIGURE 2). After oral administration of the drug in healthy subjects, chlorpropamide could be

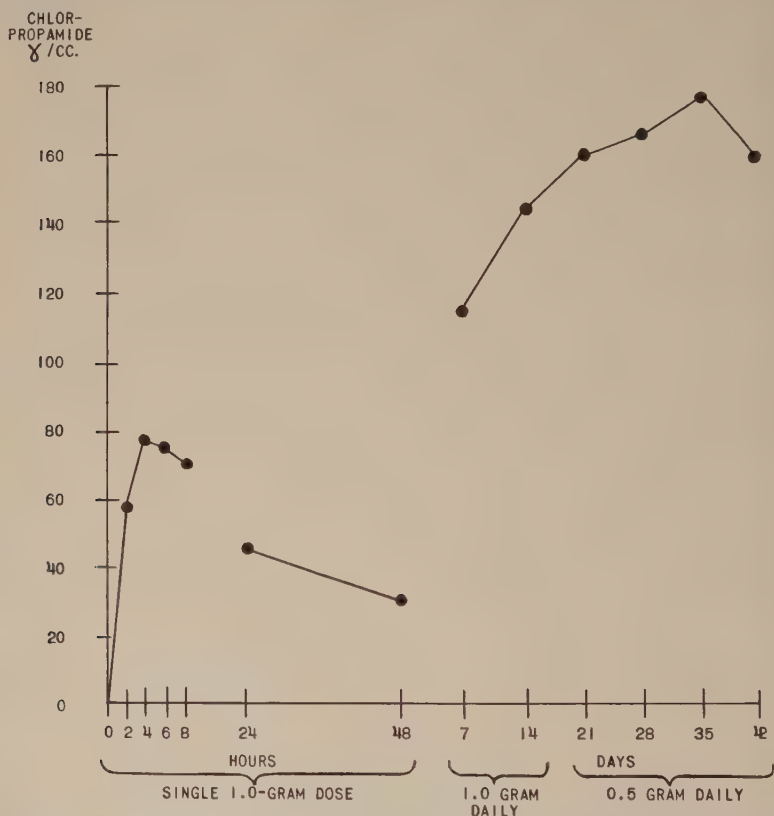


FIGURE 4. Venous levels of chlorpropamide after oral administration of the drug.

detected in the peripheral blood before any appeared in hepatic vein blood, presumably because of lymphatic absorption (FIGURE 3). In patients with hepatic cirrhosis, this relationship was altered in a manner depending upon the presence of intrahepatic and extrahepatic shunts.²

Peripheral drug levels varied considerably in different subjects after a single or repeated daily doses of chlorpropamide. A level of 40 gamma or more was usually maintained for 24 hours and was often still present at 48 hours after ingestion of 1.0 gm. of the drug (FIGURE 4). Weekly drug levels in 12 ambulatory patients showed a cumulative effect of the drug with

daily administration of 1.0 gm., and a similar pattern in some subjects receiving 0.5 gm. (FIGURE 4).

Blood sugar effects. Simultaneous blood sugar and drug levels after oral administration of chlorpropamide often showed an inverse relationship; however, the sugar response varied markedly and did not appear specifically to depend upon the height of the drug level. Although patients receiving 1.0 gm. of the drug daily often had a progressive increase in serum chlorpropamide and a decrease in blood sugar, there was usually a leveling off of both

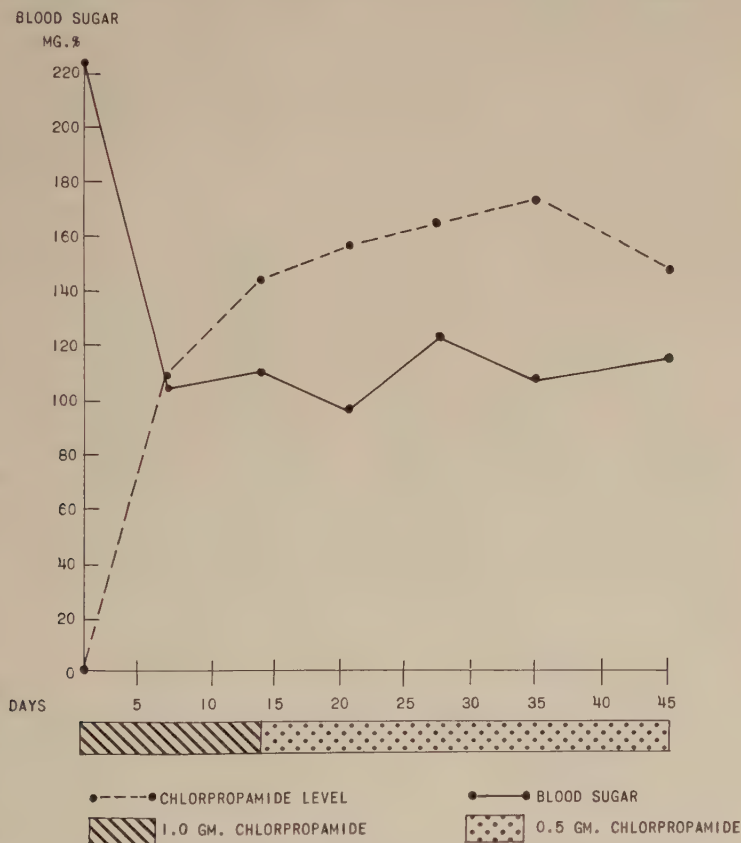


FIGURE 5. Simultaneous blood sugar and chlorpropamide levels.

measurements with continued administration of 0.5 gm. daily (FIGURE 5). No correlation of drug and blood sugar levels was demonstrable in 3 subjects who developed clinical evidence of hypoglycemia. Much higher chlorpropamide levels were noted in subjects who responded to this drug but did not develop hypoglycemia (FIGURE 6).

An earlier and greater hypoglycemic effect was noted when chlorpropamide was administered intravenously. The hepatic venous glucose level usually showed an earlier decrease than the arterial glucose, indicating a primary

hepatic action as previously demonstrated with insulin,⁶ phenethyldiguanide,⁷ and other sulfonylurea derivatives.⁸ A rebound increase in hepatic venous sugar was noted and attributed to release of epinephrine.⁶ While a significant hepatic venous arterial glucose difference was noted, little change occurred in the peripheral arterial-venous glucose difference in 6 subjects without liver disease (FIGURE 7). In contrast, 2 of 5 subjects with cirrhosis and evidence of vascular shunts exhibited a definite peripheral effect. Simultaneous chlorpropamide and blood sugar studies in 1 of these subjects showed

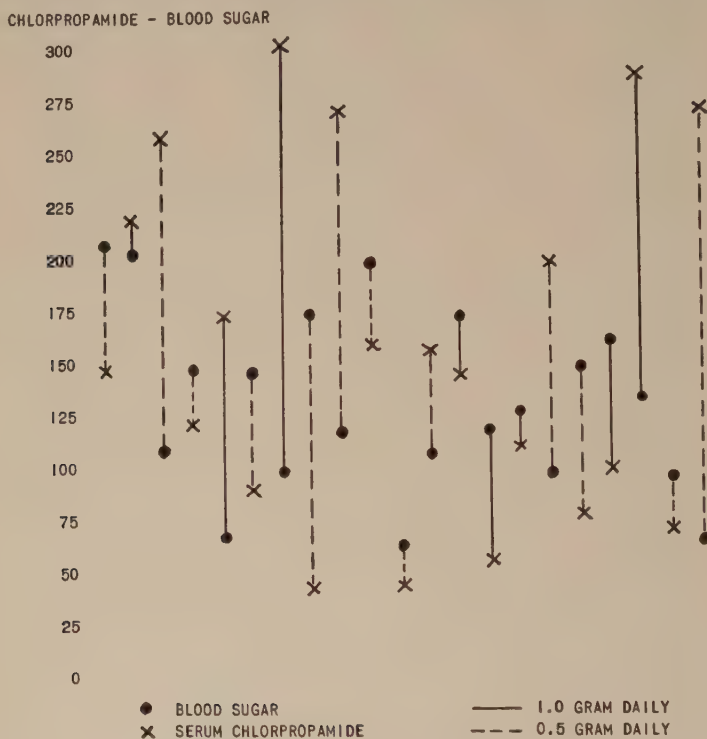


FIGURE 6. Chlorpropamide and blood sugar levels in selected diabetic patients on maintenance therapy.

diminished drug uptake by the liver, little alteration in hepatic venous glucose, and increased peripheral arteriovenous glucose difference after intravenous injection of 1.0 gm. of the drug (FIGURE 8). This phenomenon, which has numerous implications,⁹⁻¹² requires further study. Its selective nature is probably related to the location and number of shunts and the degree of liver cell dysfunction.

Effects on glucocorticoid-induced carbohydrate intolerance. Administration of glucocorticoids may cause a diabetic glucose-tolerance curve or precipitate hyperglycemia and glycosuria in patients with latent diabetes³ or liver disease.⁴ Studies were conducted on the influence of chlorpropamide on carbo-

hydrate abnormalities induced by prednisone or cortisone to determine if this drug would be of value in treating steroid-induced diabetes.¹³ Five subjects with liver disease and 1 with latent diabetes (in whom prior administration of an adrenal steroid was followed by an abnormal glucose-tolerance test reaction) were chosen for study. In each instance chlorpropamide blocked anticipated abnormalities in carbohydrate tolerance, and the post-therapy curve was frequently lower than tolerance studies without adrenal steroids.

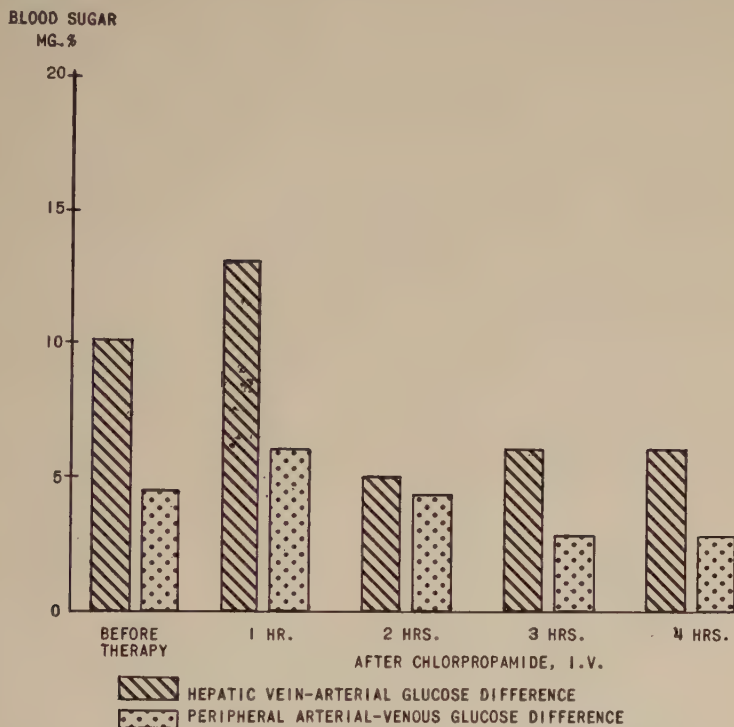


FIGURE 7. Composite of hepatic vein-arterial, and peripheral arterial-venous glucose difference in 6 patients without liver disease after administration of 1.0 gm. of chlorpropamide intravenously.

Similar results were obtained with oral and parenteral tests (FIGURES 9 and 10).

Using a different approach, data were previously collected that were interpreted as indicating that sulfonylurea derivatives do not inhibit peripheral effects of adrenocorticoid steroids.¹⁴ While it is probable that blockade of endogenously secreted steroids is not the basic mechanism of action of chlorpropamide, it is evident that in adequate dosage this drug prevents blood sugar abnormalities resulting from short-term receipt of glucocorticoids. We have also found it effective in control of carbohydrate abnormalities in a patient with latent diabetes who required prolonged adrenal steroids for a skin disorder.

Effects on epinephrine hyperglycemia. The capacity of sulfonylurea derivatives to increase liver glycogen has suggested their usefulness in treating patients with deficient hepatic glycogen resulting from diabetes or liver disease.¹⁵ Unlike healthy subjects in whom there is an increase in blood sugar of 40 mg. per cent or more after administration of epinephrine or glucagon, those with liver injury and poor glycogen stores frequently show

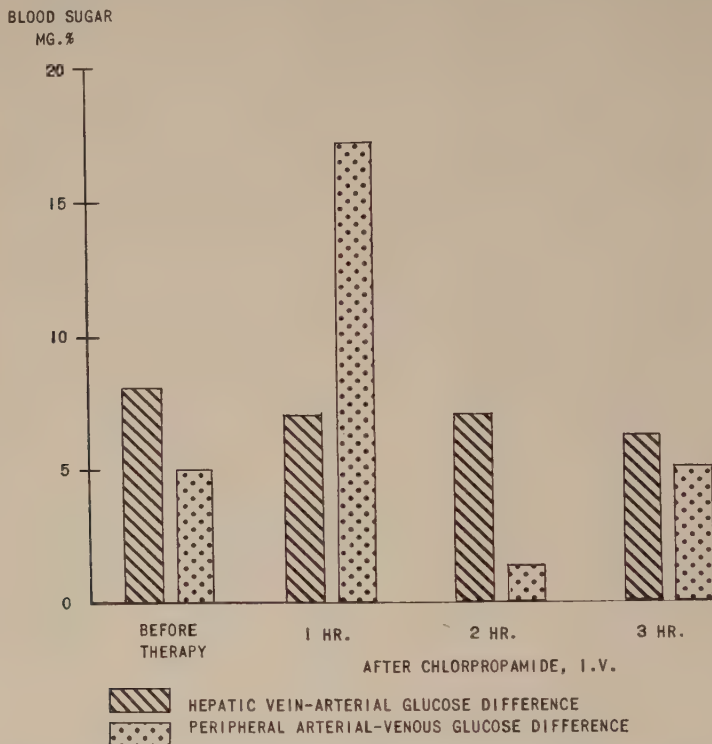


FIGURE 8. Hepatic vein-arterial and peripheral arterial-venous glucose difference in a patient with cirrhosis and vascular shunts.

a diminution in anticipated response.¹⁶ In most instances there is concomitant increase in hyperglycemic response with an improvement in biochemical and histological abnormalities of the liver. This improvement may be accelerated by glucocorticoids in nondiabetic patients and by insulin in diabetic patients. Sulfonylurea derivatives produce a similar effect in diabetes (FIGURE 10). Evaluation of the effect of chlorpropamide in 4 subjects with chronic active liver disease, normal fasting blood sugars, and decreased glycogen stores revealed a significant increase in response to epinephrine not associated with improvement in other biochemical tests in 2 patients.

Response of liver disease and diabetes. Diabetes and hepatic dysfunction frequently coexist, and assembled evidence indicates that either of these con-

ditions may be etiologically responsible for the other.¹⁵ The presence of the chloride molecule on chlorpropamide especially makes it desirable to investigate the effect of this drug on hepatic metabolism, particularly in patients with diabetes associated with liver disease. The great frequency of hepatic abnormalities in patients with diabetes most likely to respond to sulfonylurea derivatives^{6, 17} makes it difficult to evaluate hepatotoxicity. Twenty per cent

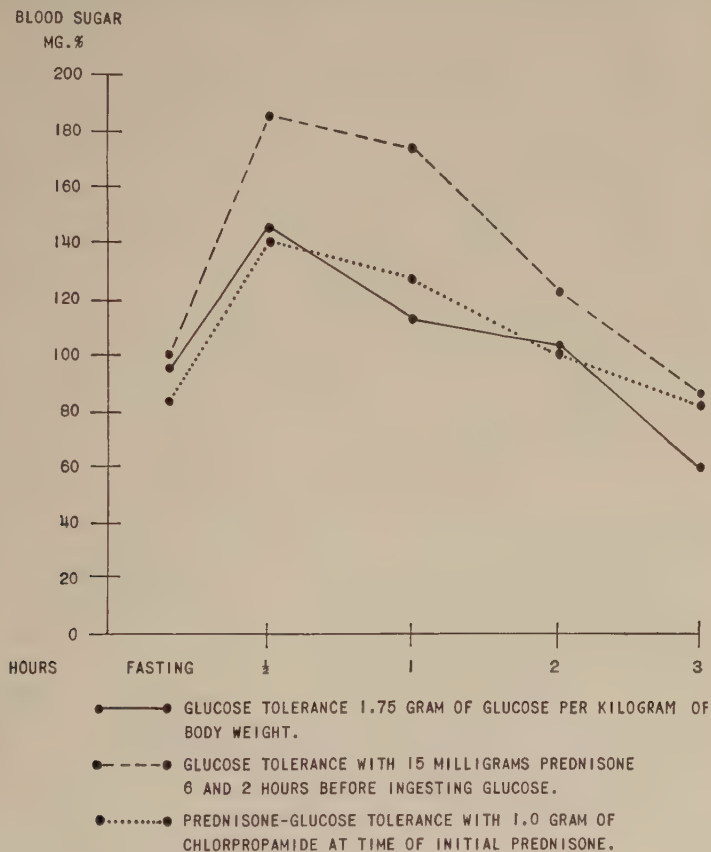


FIGURE 9. Effect of chlorpropamide on the oral adrenal-steroid glucose tolerance test.

of diabetics in this category have abnormal liver function tests, abnormal biopsies, or both in our experience. Only by long-term serial studies is it possible to evaluate hepatic effects of this drug. To date, we have not encountered any clinical, biochemical, or histological changes resulting from chlorpropamide therapy.

Many have suggested that subclinical metabolic changes involving the liver are responsible for adult onset diabetes. The association of liver injury and diabetes in hemochromatosis, pancreatitis, and other metabolic and infectious processes is well documented. The diabetic may develop

intercurrent viral hepatitis, biliary obstruction, or hepatic changes due to heart failure; however, nutritional deficiency is responsible for most instances of liver disease¹⁷ in this condition. The diabetic patient is similar to the insulin-maintained depancreatized dog, in that lipotropic deficiency produces a fatty liver and may eventually lead to cirrhosis.¹⁸ In our experience, diet and insulin or sulfonylurea derivatives have been effective in control of both

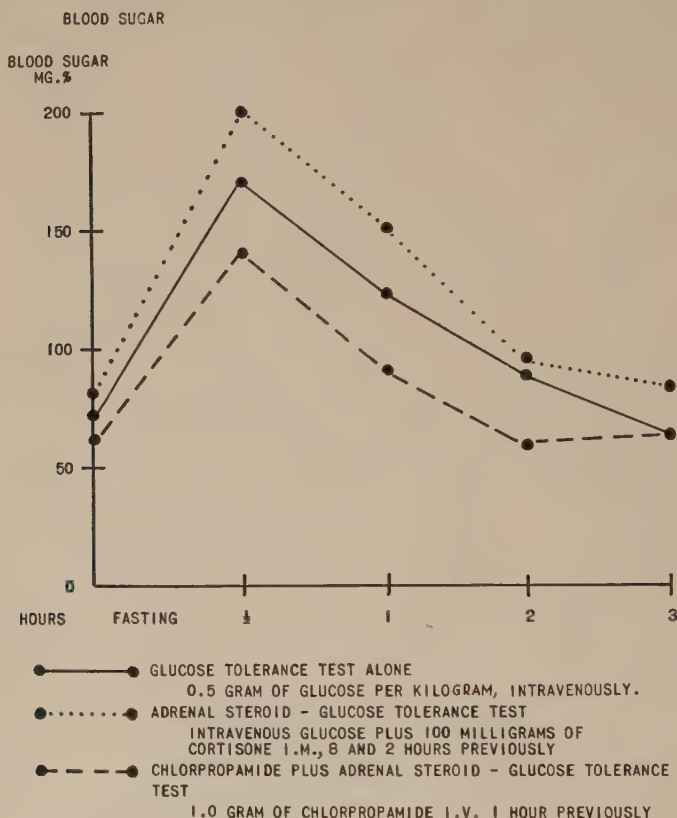


FIGURE 10. Effect of chlorpropamide on the intravenous adrenal-steroid glucose tolerance test.

the carbohydrate and hepatic abnormalities in most patients with liver dysfunction secondary to poor nutrition.

Occasionally liver disease may be responsible for a diabetic syndrome that will disappear with improvement in biochemical and histological abnormalities.¹⁹ The prednisone-glucose tolerance test, performed after disappearance of evidence of diabetes, has permitted division of such patients into a group where post-therapy adrenal steroids produce a diabetic curve, and one where glucocorticoids do not alter glucose tolerance. The mechanism of the diabetic syndrome in the latter group is unknown, but may be due to a dis-

turbance in regulation of blood sugar caused by interference with hepatic production or the use of humoral substances responsible for glycolysis and peripheral utilization of glucose.^{20, 21}

In our experience patients with this condition readily respond to carbutamide, tolbutamide, and chlorpropamide. Theoretically, sulfonylurea derivatives may be the drugs of choice in such patients because of their ease

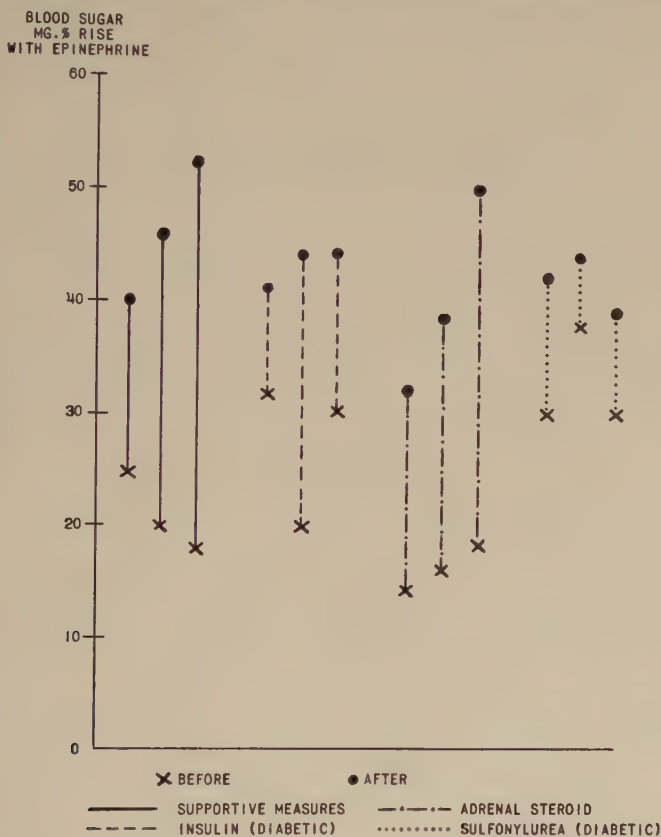


FIGURE 11. Effect of various therapeutic measures on epinephrine-induced hyperglycemia in diabetes and liver disease.

of administration and ability to increase hepatic glycogen. This is illustrated by the case of a 50-year-old laborer hospitalized because of alcoholism and tender hepatomegaly in whom prolonged follow-up has indicated the relationship of encountered hyperglycemia and glycosuria to liver injury. This patient was found to have hyperglycemia and glycosuria, which disappeared on carbutamide and a diet of 300 gm. carbohydrate, 100 gm. protein, and 100 gm. fat. There was no history of familial diabetes or clinical and laboratory evidence of pancreatitis or endocrine disorder. An initial liver biopsy showed marked fatty metamorphosis, which disappeared after

2 months of treatment. Post-therapy studies demonstrated an increase in epinephrine-induced hyperglycemia over control levels; a subsequent prednisone-glucose tolerance test was normal.

Summary and Conclusions

Chlorpropamide blood levels appear to depend primarily on its absorption and urinary excretion and, secondarily, on tissue storage and metabolism.

This drug usually produces an earlier decrease in hepatic venous glucose than arterial glucose, with little effect on peripheral arteriovenous glucose difference. Selected patients with cirrhosis and vascular shunts may exhibit a smaller than normal hepatic activity and a significant peripheral effect.

Chlorpropamide inhibits glucocorticoid-induced hyperglycemia and may be of practical use in management of patients with latent diabetes who require adrenal steroid therapy. It increases hyperglycemic response to epinephrine in diabetes and in some subjects with liver disease.

The increased incidence of liver dysfunction in diabetic patients likely to respond to sulfonylurea derivatives makes it difficult to evaluate hepatotoxicity of these drugs. They have been valuable in management of both the carbohydrate disturbance and hepatic abnormalities seen in diabetic patients with secondary hepatic changes as well as diabetes made overt by liver injury.

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CLINICAL EXPERIENCES WITH CHLORPROPAMIDE: A DOUBLE-BLIND STUDY

Charles T. Lee, Jr., Guy L. Schless, Garfield G. Duncan
Pennsylvania Hospital, Philadelphia, Pa.

To determine the clinical effectiveness of chlorpropamide as a hypoglycemic agent, a double-blind trial was conducted in the diabetic outpatient clinic at Pennsylvania Hospital. After completion of the trial, the patients were asked to continue in a follow-up study of the drug. The response to chlorpropamide as measured by the eventual state of diabetic control during the follow-up period was then compared with the results obtained during the trial and also with the control obtained with previous diabetic therapy. The results of this study indicate that chlorpropamide is a potent hypoglycemic agent that produced excellent control of diabetes in most patients.

Method

The double-blind study was divided into 3 periods of 2 weeks each. One week before starting, the patients stopped insulin or tolbutamide if they were taking either of these, but continued their regular diabetic diets. The first 2-week period of the trial was a control for all patients while, in the second and third 2-week periods, they received chlorpropamide, 1.0 gm. daily, or a placebo. The order of receiving chlorpropamide and placebo was chosen at random. Using this sequence in the study, it was possible for a patient to have no therapy other than diet for a period of 5 weeks. In selecting patients to participate in the trial, we chose only those who, we felt, could stop their accustomed treatment for this period without developing acidosis. Thus, the study was limited to the adult type of diabetic patients and did not include juvenile patients or those of known instability.

The patients came to the clinic in a fasting state at 8 A.M. and had blood drawn for a blood sugar and appropriate blood chemistries. They were then taken to a hospital dining room and were given the same 400-calorie breakfast. After breakfast they returned to the clinic and were interviewed by a physician who was unaware of the order of therapy. Two hours after breakfast, a second blood sample was drawn for sugar determination. This procedure was repeated for 6 weeks with each patient. The patients also tested their urine before meals and, at bedtime, with Tes-Tape,* recorded the results, and brought the tapes to the clinic for our count. Chlorpropamide, 1.0 gm. daily, and the placebo substance were administered identically to all patients as 2 tablets before breakfast. After the trial, patients were seen at 4-week intervals in the clinic while being continued on chlorpropamide. During the follow-up period, the dose of chlorpropamide was changed as indicated by the state of diabetic control. Postprandial blood sugars were not done during the follow-up study.

All patients on chlorpropamide had additional routine laboratory studies

* Eli Lilly and Company, Indianapolis, Ind.

performed. These comprised a complete blood count, including hemoglobin, hematocrit, white cell count, and differential count; a routine urinalysis; direct and total serum bilirubin; alkaline phosphatase; thymol turbidity; and cephalin cholesterol flocculation. These studies were done prior to beginning the double-blind trial, at the end of the trial, and routinely at 1, 2, 5, and 7 months during the follow-up period, or more often if clinical indications warranted. Blood sugar determinations were made using the Somogyi-Nelson technique, while all other studies were done by the routine methods used in the blood chemistry and hematological laboratories of the Pennsylvania Hospital.

The results of the double-blind study were assessed by averaging the fasting and postprandial blood sugars separately in every patient for each 2-week period. These values were then compared for the 3 periods. This permitted a comparison of both the fasting and the postprandial blood sugar values between the control and placebo periods, between the control and the chlorpropamide periods, and between the placebo and the chlorpropamide periods. An adequate response was taken to be a decrease in both the fasting and postprandial blood sugars of 20 per cent or more when levels in the treatment period were compared to those obtained in placebo and in control periods.

The final state of control of the diabetes achieved during the follow-up period was classified as good, fair, and poor. The criteria used were as follows: good control, fasting blood sugar under 120 mg. per cent and no glycosuria; fair control, fasting blood sugar below 150 mg. per cent and minimal glycosuria; poor control, all conditions worse than this. Those patients who had taken tolbutamide prior to this study also had their diabetic control while on that drug assessed in the same way, so that a comparison between the level of control on tolbutamide and on chlorpropamide could be made.

Results of Double-Blind Trial

Thirty-two patients completed the trial. The clinical characteristics of the group are given in TABLE 1. All patients were in the older age group,

TABLE 1
CLINICAL CHARACTERISTICS OF PATIENTS IN CHLORPROPAMIDE TRIAL

Age (years).....	59.8 av.	45-78 range
Age, onset of diabetes.....	52.6 av.	32-75 range
Duration of diabetes.....	6.6 av.	0-31 range
Previous therapy		
Insulin.....	23 patients	10-70 U. range
Diet only.....	4 patients	
Tolbutamide.....	19 patients	Just prior to trial

with an adult type of diabetes. There were 5 men and 27 women. Twenty-three had taken insulin at some time and 19 were taking tolbutamide immediately prior to the trial.

The response to chlorpropamide is shown in TABLE 2. The results are divided into 2 groups: 23 patients who received the placebo in the 2-week period before chlorpropamide, and 9 who received chlorpropamide before the placebo. Thirteen, or 56 per cent of those in the first group, showed a fall in blood sugar of 20 per cent or more when the fasting and postprandial blood sugar values during chlorpropamide administration were compared

TABLE 2
RESPONSE TO CHLORPROPAMIDE

Order of trial	No. patients	Adequate response	Inadequate response
Placebo first.....	23	13 (56%)	10 (44%)
Chlorpropamide first.....	9	8 (90%)	1 (10%)
Total.....	32	21 (65%)	11 (35%)

Adequate response = fall in fasting and postprandial blood sugar values of 20 per cent or greater compared to control and placebo levels.

TABLE 3
RESPONSE TO CHLORPROPAMIDE: PERCENTAGE CHANGE OF BLOOD SUGAR

Order of trial	Placebo Control		Chlorpropamide Control		Chlorpropamide - Placebo	
	FBS	PPBS	FBS	PPBS	FBS	PPBS
Placebo first						
Adequate response.....	+2%	+2%	-27%	-23%	-28%	-22%
Placebo first						
Inadequate response.....	+10%	+16%	+2%	+14%	-10%	+1%
Chlorpropamide first						
Adequate response.....	-22%	-19%	-33%	-31%	-11%	-10%

to the values of the placebo and the control periods. Eight of the 9 patients who received chlorpropamide first showed an average decrease in blood sugar of more than 20 per cent, but in this group the values obtained on chlorpropamide were compared only with the control values.

TABLE 3 shows the average percentage change in blood sugar values in the 2 groups. The 3 comparisons are given separately for both fasting and postprandial blood sugar levels. When placebo administration followed the control period, the blood sugar values were higher during the placebo period. However, when chlorpropamide was given before the placebo, the placebo blood sugar levels were about 20 per cent lower than the control levels. This was assumed to be due to the persistence of the hypoglycemic

effect of chlorpropamide after the drug was stopped. An adequate response in this group of patients was judged on a comparison of the control and the chlorpropamide periods only. It can also be seen that in each group the decrease in blood sugar was slightly greater in the fasting than in the post-prandial levels. However, the difference was so slight that, in a patient who has fasting hyperglycemia, determination of the fasting blood sugar alone would have been an adequate indicator of response.

Follow-Up Study

Thirty of the 32 patients continued to take chlorpropamide after the trial was completed. Of the 2 patients not followed, 1 was hospitalized with a cerebral thrombosis 5 weeks after the trial; the other refused to return. TABLE 4 gives the final results to date for the remaining 30 patients. Twenty-five of these achieved good control, although the maximum response was

TABLE 4
RESPONSE TO CHLORPROPAMIDE: FOLLOW-UP OF 30 PATIENTS

Present state of diabetic control	No. of patients	Duration until best control in weeks				
		4 or less	6	8	12	16 or more
Good: FBS under 120 mg. per cent. No glycosuria.....	25	13	3	2	6	1
Fair: FBS under 150 mg. per cent. Slight glycosuria.....	4	4				
Poor.....	1					

Of 11 patients with inadequate response during trial, 10 were available for follow-up and 9 achieved good control.

TABLE 5
RESPONSE TO CHLORPROPAMIDE: COMPARISON OF CONTROL WITH TOLBUTAMIDE AND CHLORPROPAMIDE

Tolbutamide control		Chlorpropamide control		
		Good	Fair	Poor
Good.....	7	7	0	0
Fair.....	7	7	0	0
Poor.....	5	4	1	0
Total.....	19	18	1	0

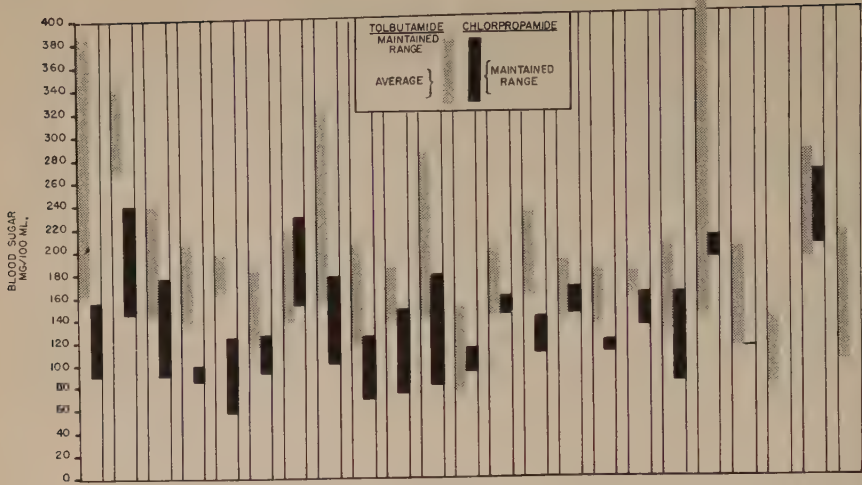


FIGURE 1

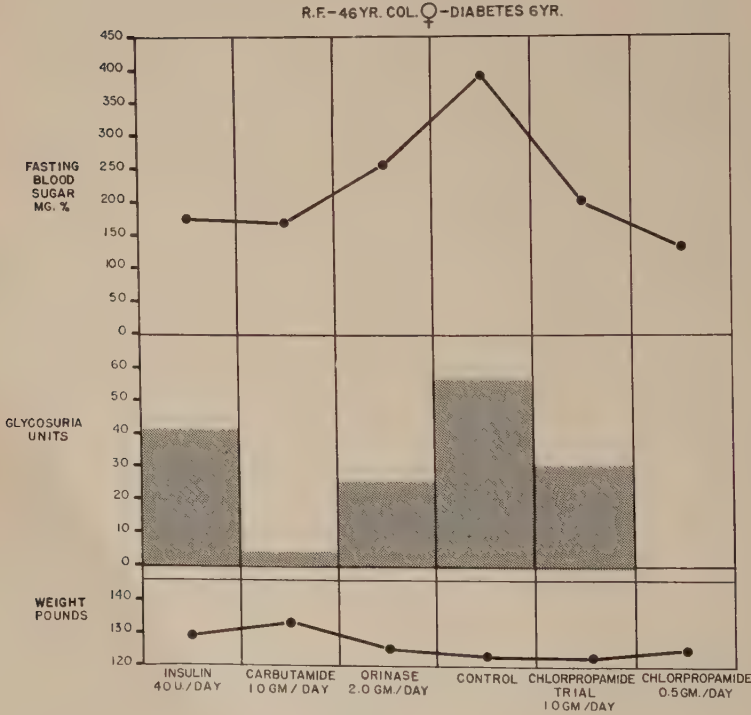


FIGURE 2

often delayed for several weeks. Probably this was due to the fact that the dose of chlorpropamide was usually reduced to 0.5 gm. daily after the trial proper was completed. The 1 patient who failed to show a good response was a 45-year-old Negro female with diabetes of 5 years' duration who had required 50 U. of insulin daily for indifferent control before the study.

Nineteen patients were taking tolbutamide before the chlorpropamide trial started. The effectiveness of diabetic control with these 2 drugs is compared in TABLE 5. Seven patients showed good control with both drugs.

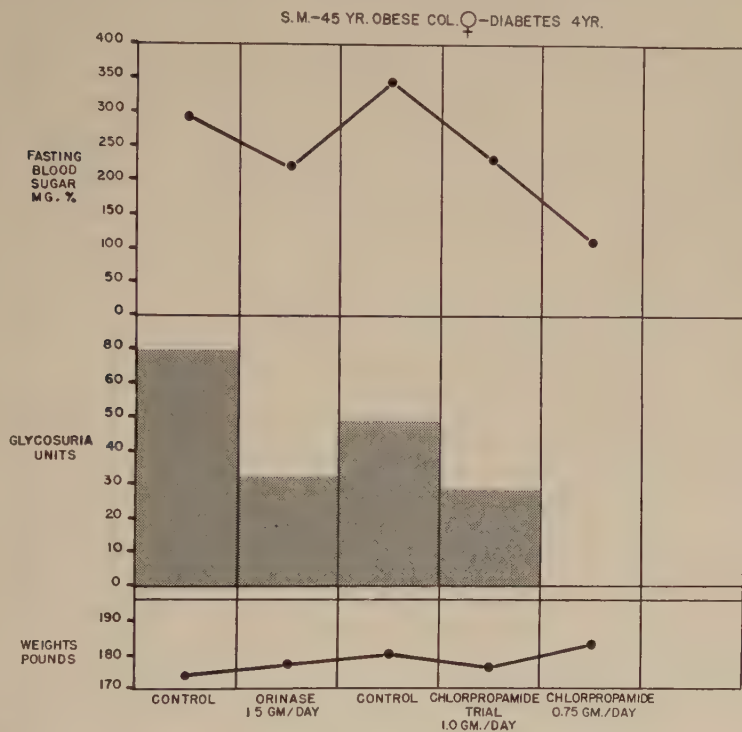


FIGURE 3

Of 12 patients not well controlled on tolbutamide, all showed improvement with chlorpropamide, and with one exception, the final state of diabetic control was very satisfactory. FIGURE 1 illustrates this by comparing the range of fasting blood sugar determinations on the two drugs. In almost every case the blood sugar values are substantially lower with chlorpropamide. There was no correlation between success or failure with tolbutamide and the dose of chlorpropamide necessary to achieve good control. FIGURE 2 presents the chronological history of a patient who was treated successively with insulin, carbutamide, tolbutamide, diet alone, and chlorpropamide. Average values for fasting blood sugar, glycosuria, and weight are given for each period of therapy. The patient showed poor control on 2.0 gm. tolbut-

amide daily, but has been very effectively controlled with a single daily 0.5 gm. dose of chlorpropamide. FIGURE 3 shows the results of oral therapy in a gluttonous woman who might be controlled by a low-calorie diet and weight reduction. Neither threats nor bribes nor tolbutamide was effective in controlling her appetite or her diabetes; however, on a dose of chlorpropamide that has now been reduced to 0.5 gm. daily, she has an average fasting blood sugar of 100 mg. per cent and no glycosuria. Her appetite is unchanged and she continues to gain weight.

Toxicity

The side effects and possible toxic reactions noted with chlorpropamide are listed in TABLE 6. Six patients complained of symptoms suggestive of

TABLE 6
RESPONSE TO CHLORPROPAMIDE: SIDE EFFECTS AND TOXIC REACTIONS

Side effect or reaction	No. of patients
Hypoglycemic symptoms.....	8
Fasting blood sugar below 60 mg. per cent.....	4
Hunger.....	5
Gastrointestinal disturbance.....	0
Skin rash (transient).....	2
Liver function test abnormal (transient).....	3
Blood count or urine tests abnormal.....	0

hypoglycemia and, in 4 instances, fasting blood sugar values below 60 mg. per cent were found. One patient was hospitalized briefly with hypoglycemic coma and a blood sugar level of less than 40 mg. per cent after taking 1.0 gm. chlorpropamide daily for 3 weeks. Significant hypoglycemia is a definite hazard with chlorpropamide therapy. Two patients developed a transient itching maculopapular eruption. There was no associated eosinophilia and the rashes disappeared with symptomatic treatment despite continuing chlorpropamide. No patient showed a significant change in the blood count or in urinalysis. In 3 patients, the alkaline phosphatase became abnormally elevated after several weeks of therapy, but fell to normal spontaneously while therapy with chlorpropamide was continued.

Summary

Thirty-two patients participated in a double-blind trial of chlorpropamide. All had an adult type of diabetes, but every patient also had glycosuria and fasting hyperglycemia during the control period of no therapy. Twenty-one, or 65 per cent of the group, had a decrease in both fasting and postprandial blood sugar levels of more than 20 per cent during the trial. Thirty patients continued chlorpropamide after the trial and 25 achieved good control of the diabetes. The average maintenance dose of chlorpropamide was 0.5 gm.

or less daily given in a single dose. The maximum response to chlorpropamide may be delayed until 4 weeks or more after therapy is started. Comparison of diabetic control between tolbutamide and chlorpropamide in the same patients shows that good control often can be achieved with chlorpropamide when tolbutamide has been proved ineffective. Chlorpropamide was a more potent hypoglycemic agent than tolbutamide. Toxic reactions were seen in 5 patients, but in every case the manifestation of toxicity subsided without stopping therapy. Hypoglycemic reactions, occasionally severe, may be provoked by chlorpropamide, and the dosage must be individually adjusted.

Acknowledgments

We thank Brooks W. Gilmore, Joyce Laubach, and Sandra Frank for their invaluable assistance during the study.

LONG-TERM THERAPY WITH CHLORPROPAMIDE

A. W. Alvi and J. P. Maclean

Department of Clinical Investigation, St. Boniface Hospital, and Department of Physiology and Medical Research, University of Manitoba, Winnipeg, Canada

The influence of chlorpropamide has been evaluated in 56 diabetic patients with reference to changes in hyperglycemia, glycosuria, liver function, the hematopoietic system, and general health.

Materials and Methods

The subjects were selected largely from the diabetic clinic of St. Boniface Hospital. As a group they tended to have little respect for dietary restrictions. Before inclusion in the study an attempt was made to categorize the diabetes as stable (adult, obese, nonketotic, and senile) or labile (juvenile, thin, ketotic, and brittle). Using this clinical assessment, we felt that 49 subjects had stable diabetes.

The initial response to chlorpropamide was assessed in hospital on 37 patients. The other 19 were studied as outpatients. The laboratory tests done prior to therapy and at intervals thereafter were: a complete urinalysis; hemogram; oral glucose tolerance (Folin-Wu); blood urea nitrogen; plasma prothrombin time; serum alkaline phosphatase (King-Armstrong), glutamic oxaloacetic acid transaminase, phosphohexose isomerase, thymol turbidity and flocculation, cephalin cholesterol flocculation, cholesterol, and esters; and Bromsulphalein excretion. The radioactive iodine uptake by the thyroid was measured in 5 of these subjects.

Depending on the type and severity of the diabetes, chlorpropamide administration was begun in one of several ways: the drug was given and the insulin gradually reduced; insulin was discontinued 24 hours before giving chlorpropamide; insulin was discontinued for several days; patients who had not been using insulin were given the drug after a preliminary assessment on a constant diet. In all cases 1.0 gm. chlorpropamide was given the first day and 0.5 gm. daily until the hypoglycemic effect could be evaluated. The dose was then adjusted to achieve optimum fasting blood sugar levels. A stable diabetic was not considered resistant to chlorpropamide unless he failed to show a decrease in hyperglycemia on 2.0 gm. daily.

Results and Discussion

Thirty-nine of the 56 subjects have received chlorpropamide for at least 4 months, which is our criterion for long-term therapy. The 7 patients adjudged to have labile diabetes all failed to show a hypoglycemic effect with chlorpropamide either alone or in combination with insulin. They were given the drug from a minimum of 4 days to a maximum of 20 days. We concluded that chlorpropamide, like the other sulfonylurea drugs, does not control the labile diabetic. However, our dose schedule for initiating therapy with chlorpropamide was not varied, and it is possible that a higher initial

TABLE 1
ANALYSIS OF PATIENTS WITH STABLE DIABETES

Age and sex	Diabetes		Insulin			Chlorpropamide			Comments
	Duration (years)	Complications	Duration (years)	Daily dose (units)	Control*	Duration (days)	Dose (gm.)	Control*	
56M	New	+	0	0		7-20†	0.5	G‡	Sudden death, M. infarct
74M	2	+	Only with acute cholecystitis			10	0.5	G‡	Recurrent cholecystitis
66M	1½	-	0	0	F	7	0.5	†‡	Obstructive jaundice due to Ca. pancreas
70M	12	+	0	0		20	0.75	G‡	
46F	4	-	0	0	P	40	0.5	†‡	P on 0.5 gm.
47F	6	+	0	0	P	45	0.75	†‡	P on 0.75 gm.
56M	9	++	0	0	P	7	2.0	P	
68F	14	-	14	75	P	21	2.0	P	
65M	16	+	12	40	P	10-16†	2.0	P	Diarrhea first course—none later
66F	10	-	10	30	G	14	0.75	P	Anorexia, drowsy, ? reaction
66F	5	+	5	70	P	120	1.5	P	Hypoglycemic effect in hospital
45F	9	-	4	35	P	200	2.0	G	Initial success-operation
42F	3	-	3	30-40	F	270	1.0	G-P	Initial success
72F	10	+++	5	25	P	240	1.0	G	Died postop. cholecystectomy
41M	1½	-	1½	35	G	90	0.5	G	Triamcinolone 100 mg./day died leukemia
59F	19	+	0	0	F	300	0.5	G	
81F	2	+	½	15	F	180	0.25	G	
71F	7	+	5	30	P	180	0.75	F	Gangrenous toe
63F	23	++	15	30	F	180	0.5	G	Hyperthyroidism
76F	2	++	1½	15	G	120	0.5	G	Cellulitis
66F	3	-	3	50	P	150	0.5	G	
72M	5	++	0	0	F	270	0.25	G	Transurethral resection
63F	9	+	1§	25	F	240	0.75	G	Pneumonia
75F	22	-	18	30	G	270	0.25	G	
71F	5	+	5	30	P	240	1.0	G	
52M	2	-	0	0	P	270	0.5	F	Electroshock therapy
74M	6	+	0	0	P	270	0.75	G	
49F	5	-	2	30	P	150	1.0	F	
65M	1	+	0	0	P	180	0.5	G	
68F	8	++	4	20	F	270	0.25	G	
79M	5	+	4	30	F	240	0.5	G	
50F	7	+	5	50	P	270	0.75	F	Myocardial infarct.
62F	5	+	¾	30	P	240	0.5	G	Abdominal burn
49F	1	-	0	0	F	180	0.25	G	Cerebrovascular accident
77M	?Recent	¾	¾	35	F	180	0.5	F	
77F	5	+	5	35	F	135	0.25	G	
70F	16	++	0	0	F	120	0.25	G	
67F	1	-	⅙	25	F	300	0.25	G	
55F	5	++	5	10-30	F	180	0.25	G	
74F	12	++	0	0	P	130	0.75	G	
50F	7	-	2	15	F	240	0.5	G	
63F	2	++	¼	35	G	200	0.25	G	
70F	7	+	6	80	P	180	1.5	F	Carcinoma, colon resected
67F	?	-	4	20	P	240	0.5	G	
64F	8	+	2½	35	P	210	0.75	G	
73F	3	+	0	0	F	190	0.25	G	
60F	?	+	⅓	15	F	270	0.25	G	
64F	6	-	0	0	F	210	0.25	G	
65F	New	-	0	0		120	0.5	G	

* Control: P = poor, F = fair, G = good.

† Chlorpropamide given in 2 courses.

‡ Lost to follow-up.

§ At onset of diabetes.

dose might have produced hypoglycemia. No side effects other than ketosis were observed. One labile patient, a 39-year-old female, died 10 days after chlorpropamide was discontinued. The cause of death was a pulmonary embolus following electroshock therapy. The consulting psychiatrist felt that her psychosis was not drug induced.

Some of the pertinent data on the 49 stable diabetics are shown in TABLE 1. The complications indicated as +++ refer to retinopathy, neuropathy, nephropathy and other occlusive vascular disease; by good control we mean

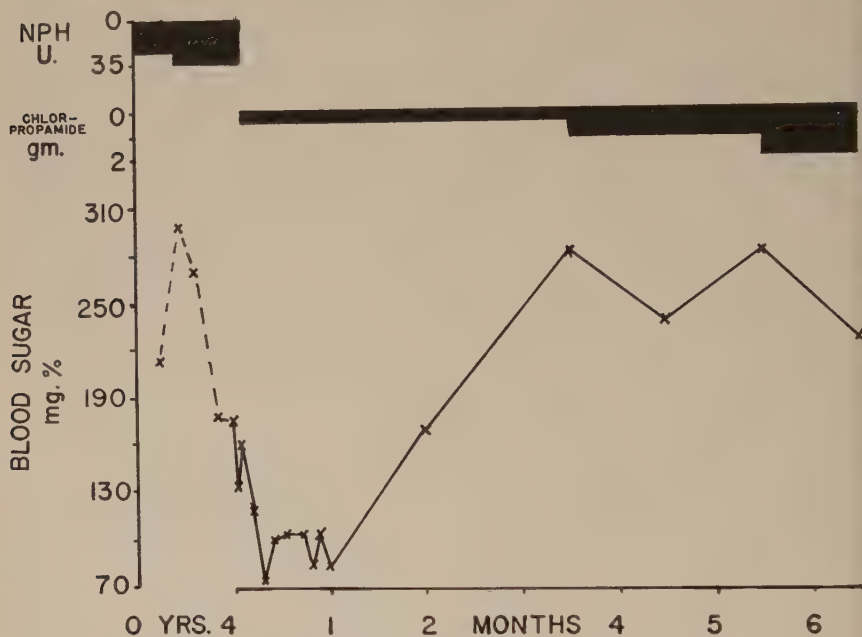


FIGURE 1. Patient H. M., female, aged 45, diabetic for 9 years.

aglycosuria and fasting normoglycemia, and by fair control we mean fasting blood sugars (Folin-Wu) between 120 and 160 mg. per cent. Fasting blood sugars over 160 mg. per cent indicate poor control. The patients varied in age from 41 to 81 years, with a mean age of 63. Ten of these subjects did not satisfy our criterion for long-term therapy. Six were lost to follow-up; 3 failed to show a hypoglycemic effect on 2.0 gm. chlorpropamide daily; and 1, who remained hyperglycemic on 0.75 gm. daily, became anorectic and drowsy. Of those lost to follow-up, 1 died suddenly of a myocardial infarction diagnosed post-mortem. His initial response had been good and he may have been hypoglycemic at the time of infarction. One died of *E. coli* septicemia during his third bout of acute cholangitis. He was normoglycemic on 0.5 gm. chlorpropamide between the second and third attacks. The drug was discontinued at the onset of symptoms 2 weeks prior to death. At post-mortem, multiple liver abscesses were found, together with common duct calculi and a contracted gall bladder. A third patient developed

jaundice of the obstructive type 7 days after initiation of chlorpropamide therapy, and the drug was discontinued. A diagnosis of carcinoma of the pancreas was made at laparotomy. Of the other 3 patients lost to follow-up, 1 showed good initial control on 0.5 gm., and 2 showed poor initial control on 0.5 gm.

All of the 39 long-term subjects showed an initial hypoglycemic effect. Three were late failures. FIGURE 1 shows the decrease in fasting blood sugar level in a 45-year-old obese female who was changed from insulin to

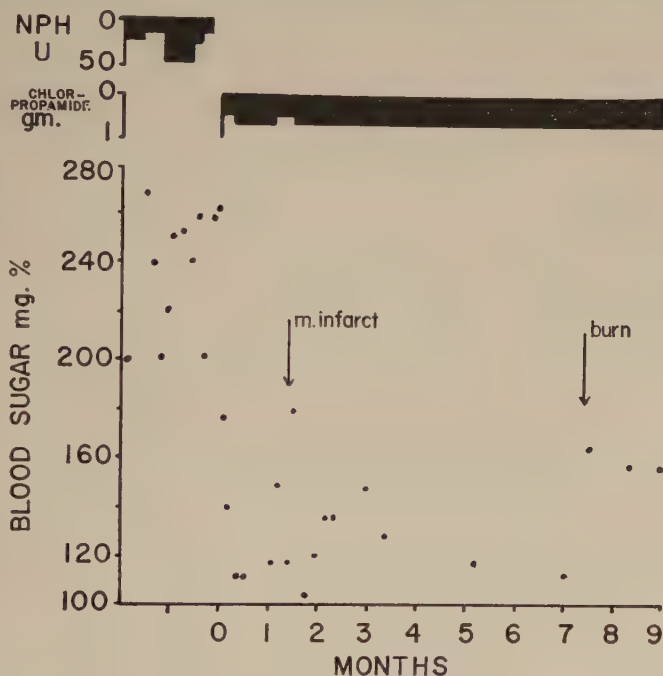


FIGURE 2. Patient D. M., female, aged 50, diabetic for 7 years.

chlorpropamide while maintained on a constant diet. After discharge from hospital her blood sugar rose to previous levels, and she gained weight. Six other patients were not as well controlled at home as they had been in hospital, but control at home was better than on previous therapy. The other 30 patients remained well controlled at home.

Two of the long-term subjects have died, one of leukemia and the other of subphrenic abscess following cholecystectomy for chronic recurrent cholecystitis. Both patients showed good control on chlorpropamide. Good control was maintained on the leukemic patient during a 3-week period when he received triamcinolone (100 mg./day).

In the long-term group 9 further medical events have occurred: 1 myocardial infarct, 3 operations, 1 gangrenous toe, 1 cellulitis, 1 cerebrovascular accident, 1 bronchopneumonia, and 1 continuing hyperthyroidism. Chlorpropamide was continued in each, and ketosis did not develop. FIGURE 2

shows the blood sugar response in a 50-year-old female who had first a myocardial infarct and later a second-degree scald of the abdomen. The possible role of chlorpropamide in the production of these fatal and nonfatal events is not known, but no direct correlation could be detected.

Oral glucose tolerance tests done 24 hours following therapy showed a hypoglycemic effect, but no other difference in the type of curve. FIGURE 3 shows 1 pretreatment and 3 treatment curves in a 52-year-old male. The subject was on an unrestricted diet during the 7 months between the third and fourth tests. The fourth curve shows less hypoglycemic effect.

Liver function showed no deterioration except in 3 patients who died. In those 3 a satisfactory explanation of the pathology was found (see above).

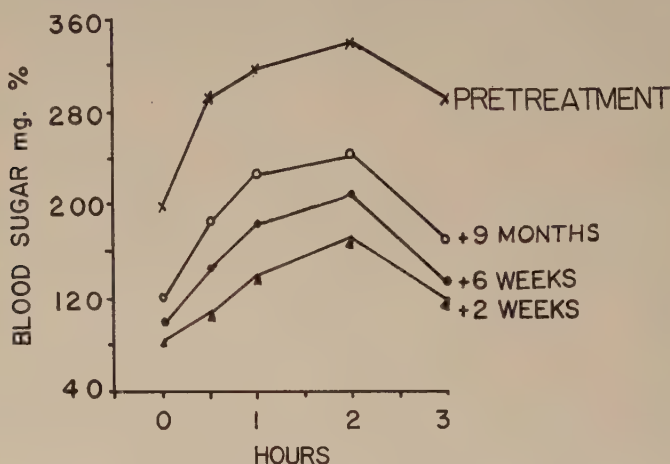


FIGURE 3. The effect of chlorpropamide on oral glucose tolerance. Patient A. K., male, aged 52, diabetic for 2 years.

In 3 other patients Bromsulphalein excretion improved during drug administration. Hematological studies showed no change in hemoglobin and no evidence of agranulocytosis or thrombocytopenia. Two patients showed a transient unexplained eosinophilia. Chlorpropamide did not change the uptake of radioactive iodine by the thyroid in the 5 patients studied, including the one with hyperthyroidism.

Symptoms that may have been due to the drug were diarrhea in one patient and pruritus in another. In both cases the drug was continued, with disappearance of the complaint. One patient complained of anorexia and drowsiness with hyperglycemia but no acidosis, and the drug was discontinued.

An attempt was made to find a correlation between duration of insulin therapy and blood sugar response to chlorpropamide in the stable group. Of 18 who had never taken insulin, 10 showed a good response, 1 a fair response, and 1 a poor response. Six were lost to follow-up. Of 18 on insulin less than 5 years, 14 showed a good response, 2 a fair response, and 2 were late failures. Of 13 on insulin for 5 years or more, 6 showed good control, 3 showed fair control, and 4 showed poor control. Of the 31 who

had used insulin, 5 had taken more than 40 units daily. Three of these patients showed improved control with chlorpropamide. Ten patients in the stable group had previously used tolbutamide. Of these, only 3 had improved on tolbutamide, whereas 7 improved on chlorpropamide. This difference in response might be explained by the greater attention given to the patients during the chlorpropamide trial.

The maintenance dosage of chlorpropamide for the 36 well-controlled long-term patients varied from 0.25 to 1.5 gm. daily. Thirteen used 0.25 gm., 13 used 0.5 gm., 6 used 0.75 gm., 3 used 1.0 gm., and 1 used 1.5 gm. daily. Final adjustments in dosage have not yet been made.

Summary

We could neither control the hyperglycemia nor prevent ketosis with chlorpropamide in 7 labile diabetics. Thirty-six of 43 stable diabetics were controlled better with chlorpropamide than with previous therapy. Although the mortality and morbidity rates were high, chlorpropamide could not be implicated. No deterioration of liver, hematopoietic, or thyroid function could be determined. Diarrhea, pruritus, and anorexia each occurred in one patient. Neither the amount nor the duration of insulin therapy correlated with the hypoglycemic response to chlorpropamide. Some tolbutamide failures were controlled by chlorpropamide. The maintenance dosage of chlorpropamide averaged 0.5 gm. daily.

Acknowledgments

We acknowledge with pleasure the help of M. C. Blanchaer and Paul Green, staff members, St. Boniface Hospital, Winnipeg, Canada.

CLINICAL EVALUATION OF CHLORPROPAMIDE IN DIABETES MELLITUS

Ismael Canessa, Sergio Valiente, Iris Mella

Departamento de Nutrición y Diabetes, Servicio de Medicina Profesor Armas Cruz, Hospital San Juan de Dios, Santiago, Chile

The use of the sulfonylureas for the treatment of diabetes mellitus is now well accepted. Numerous studies dealing with the indications, contraindications, and clinical results of oral treatment with carbutamide and tolbutamide have been published. Therefore, in a clinical study of a new drug, chlorpropamide, 1-propyl-3-(*p*-chlorobenzenesulfonyl)urea, it seems unnecessary to survey the literature, even for the sake of comparison, since the fundamental therapeutic data are so well known.

MATERIALS

Chlorpropamide was administered to 80 diabetics (38 males, 42 females). The ages of the patients, dates of onset, duration of diabetes, nutritional

TABLE 1
AGE AND NUTRITIONAL STATUS IN 80 PATIENTS TREATED WITH CHLORPROPAMIDE

Nutritional status	Age in years			Total	Percentage
	<20	21-40	>40		
Obese.....	—	4	44	48	60.0
Normal.....	—	5	9	14	17.5
Underweight.....	2	5	11	18	22.5
Total.....	2	14	64	80	100.0

TABLE 2
AGE OF ONSET AND YEARS OF DIABETES IN 80 PATIENTS TREATED WITH CHLORPROPAMIDE

Age of onset	Years of diabetes					Total	Percentage
	<1	1-5	6-10	11-15	16-20		
<20.....	—	2	—	—	—	2	2.5
21-40.....	3	8	8	—	4	23	28.7
>40.....	6	19	12	15	3	55	68.7
Total.....	9	29	20	15	7	80	100.0

TABLE 3
COMPLICATIONS OF DIABETES AND ASSOCIATED DIAGNOSES IN 80 PATIENTS TREATED
WITH CHLORPROPAMIDE

Complications or associated diagnoses	No. of cases
Retinopathy.....	29
Nephropathy.....	17
Atherosclerosis.....	42
Blood hypertension.....	15
Tuberculosis.....	9
Other diagnoses.....	10
Patients with 1 or more complications or associated diagnoses.....	53
Patients without complications or associated diagnoses.....	27

TABLE 4
THERAPY PRIOR TO CHLORPROPAMIDE IN 80 PATIENTS

Prior therapy	No. of cases	
Insulin treatment.....		61
Insulin only.....	35	
Insulin and oral treatment (nonsimultaneous).....	26	
Oral treatment only.....		8
New cases without treatment.....		4
Old cases without treatment.....		7
Total.....		80

TABLE 5
YEARS OF INSULIN AND DOSE IN 61 PATIENTS TREATED WITH CHLORPROPAMIDE

Years of insulin	Dose of insulin (units)			Total
	<20	21-40	>41	
<1.....	3	11	4	18
1-5.....	4	15	1	20
6-10.....	—	8	1	9
11-20.....	1	5	1	7
Not determined.....	3	4	—	7
Total.....	11	43	7	61

condition, complications and associated maladies, and previous treatment are illustrated in TABLES 1, 2, 3, 4, and 5.

METHOD

Thirty-four cases were first treated while hospitalized; all the other cases were outpatients. The diet, adjusted to the theoretical caloric requirements, varied between 150 gm. and 250 gm. of carbohydrates. The initial dosage of chlorpropamide was 2 gm. (23 cases) and 1.5 gm. (5 cases) daily, based on the usual dosage schedules for the other sulfonylureas. As our evaluation continued, we found that we could reduce the initial dosage to 1 gm. (49 cases) and 0.5 gm. (3 cases) daily. In general, divided doses were employed, except during the maintenance period, when the dose was reduced to 0.25 gm. daily. In the case of 2 patients, both chlorpropamide and insulin were given.

The outpatients were examined weekly at first, and then monthly. In these cases the blood sugar was determined during a fasting state and urine sugar was determined from a 24-hour pooled sample (glycemia determined by the Solomos method). Before and during the course of treatment tests were made of hepatic, hematopoietic, and renal functions (floculation, alkaline phosphatase, prothrombin, Bromsulphalein retention, hemogram, and complete urine tests).

The first dose was administered as a single dose and the hypoglycemic action of the drug was determined in 76 cases at 3, 4, 5, and 24 hours (selection test). On the basis of these results the potency of the drug was established. The patients fasted during the first 5 hours of study. In addition to observing immediate action, we wished to determine whether the results of this test would relate to the results of previous treatment. Serum chlorpropamide also was tested during the course of the treatment.*

RESULTS

Hypoglycemic Action of the Drug

Selection test. Response as observed in the selection test was based on blood sugar determinations on samples taken 5 hours after the initial dose was administered (final glycemia). On the basis of our prior experience with other sulfonylureas,¹ the result was classified as good when the final blood sugar was lower than 1.60 gm. per cent or a drop of more than 50 per cent from the initial figure occurred. The result was considered fair when the blood sugar was between 1.61 and 2 gm. per cent or when a drop of between 25 and 50 per cent from the initial figure occurred; the result was termed poor when the final blood sugar was over 2 gm. per cent or when a decrease of less than 25 per cent from the initial figure occurred.

A significant hypoglycemic effect appeared in 52 patients (68.4 per cent); the other 24 did not respond to the drug. FIGURE 1 presents the curves of the average values obtained in the patients who responded to and those who did not respond to the drug. The tests carried out with 1 and 2 gm. of chlorpropamide are shown separately. The greatest decrease in blood sugar was

* Using the technique communicated to us by Chas. Pfizer & Co. Inc., Brooklyn, N. Y.

noted after 5 hours (1.09 and 1.38 gm. per cent with 2 gm. and 1 gm. respectively). A longer period of fasting was not feasible and we do not know whether we should have obtained lower values if the observation had been prolonged. After 24 hours, the blood sugar value usually is lower than its initial value. The results presented in this figure demonstrate that, like the other sulfonylureas, chlorpropamide has an immediate hypoglycemic action.

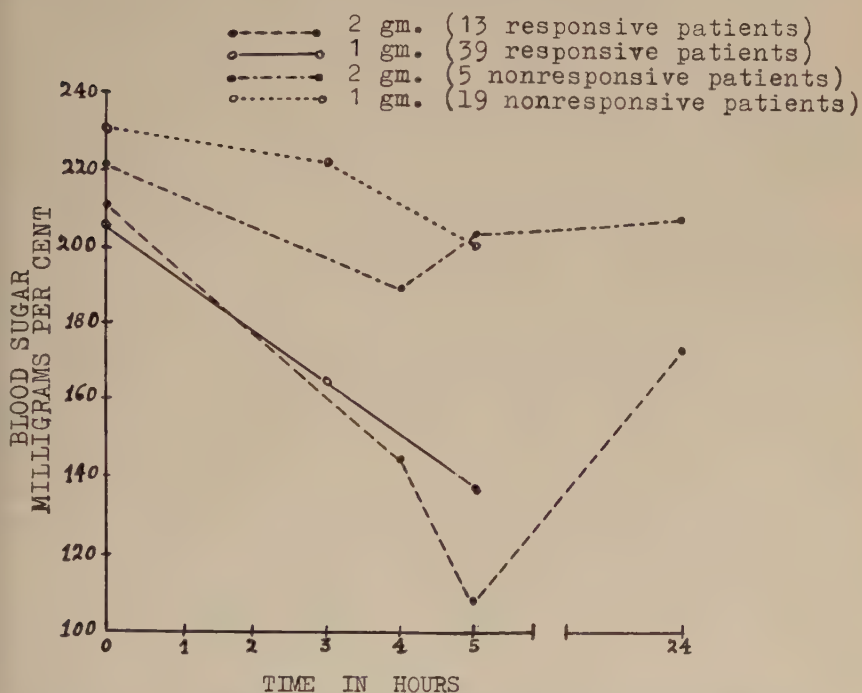


FIGURE 1. Average blood sugar response to different doses of chlorpropamide.

TABLE 6 presents the results of four selection tests conducted on patients who received chlorpropamide, in which serum levels of the drug were also determined at the same time.

It may be seen that in the 3 cases with good hypoglycemic response there were considerable quantities of chlorpropamide in the blood during the early hours of the test. These values increased even more at 5 hours. The increase is more rapid in some cases. There seems to be a period of latency of action, since the blood sugar values rapidly fall at 5 hours after 0.5 or 1 gm. are administered, even though in the serum at the 3-hour determinations there are greater quantities of chlorpropamide than are usually observed in patients who have responded to the drug and are receiving maintenance dosage. The case with poor response (case 4) showed serum chlorpropamide levels at 3 and at 24 hours that were higher than those obtained in other patients who had a favorable response. In addition to the usual differences

TABLE 6
CHLORPROPAMIDE SERUM LEVEL AND BLOOD SUGAR RESPONSE IN 4 PATIENTS,
AFTER ADMINISTRATION OF A SINGLE DOSE OF 1.0 GM.

	0 Hour blood sugar	3 Hours		5 Hours		24 Hours	
		Blood sugar	Serum chlorprop- amide mg. %	Blood sugar	Serum chlorprop- amide mg. %	Blood sugar	Serum chlorprop- amide mg. %
Case 1....	2.22	1.66	4.03	1.14	8.21	1.25	6.59
Case 2....	1.66	1.53	3.90	1.43	5.30	1.90	9.89
Case 3....	3.08	2.00	6.40	1.29	9.23	Not done	Not done
Case 4....	2.10	2.67	7.41	1.90	Not done	2.85	6.78

Case 1: Record 492 (E. R.)

Case 2: Record 509 (C. Z.)

Case 3: Record 501 (E. M.)

Case 4: Record 381 (P. V.)

TABLE 7
TIME OF CHLORPROPAMIDE TREATMENT REQUIRED TO OBTAIN CONTROL OF BLOOD
SUGAR OR GLYCOSURIA*

Time of treatment	Blood sugar			Percentage	Glycosuria			Percentage
	No. cases		Total		No. cases		Total	
	2 gm.	1 gm.			2 gm.	1 gm.		
1st Week	9	23	32	58	9	22	31	58.4
2nd Week	5	12	17	31	2	11	13	24.5
3rd Week	2	2	4	7.3	—	1	1	1.9
4th Week	—	—	—	—	1	3	4	7.5
5th Week	1	1	2	3.6	1	3	4	7.5
Total	55 Cases			99.9	53 Cases			99.8

* Blood sugar below 150 mg. per cent; no sugar in 24 hour urine specimen.

in response that can be expected due to the different degrees of diabetes, there also may be variations caused by differing rates of absorption and excretion.

Normalization of blood sugar and disappearance of sugar from the urine. Blood sugars were returned to normal levels in 55 of the 80 patients. Of these, 58 per cent occurred in the first week (16 per cent in the first 4 days), and 31 per cent in the second week. Disappearance of sugar from the urine occurred at similar times (TABLE 7). There was no evident difference

between those cases receiving 2 gm. or those receiving 1 gm. during the initial treatment period. Compensation was somewhat more prompt in the hospitalized cases than in the outpatients, possibly because of better observance of the diet.

Clinical Results

Results are classified as good, fair, and poor. This is the same standard as is used in other publications of this department, with the omission of the category excellent, no curves having been taken for postprandial blood sugar during the day (TABLE 8).

TABLE 8
STANDARD OF DIABETIC CONTROL*

Control	Fasting blood sugar mg. per cent	24 Hours glycosuria gm.	2 Hours postprandial blood sugar mg. per cent
Excellent.....	<150	0	<180
Good.....	<150	0	<225
Fair.....	150-200	<20	<250
Poor.....	>200	>20	>250

* 180 to 200 gm. of carbohydrates in the diet.

TABLE 9
RESULTS OF CHLORPROPAMIDE TREATMENT IN 80 PATIENTS

Results	No. Cases	Percentage
Good.....	44	55.00
Fair.....	23	28.75
Poor.....	13	16.25
Total.....	80	100.00

The results have been analyzed in relation to several factors: the age of the patients; age of onset of the disease; duration of the disease; the patient's nutritive state; complications and associated disorders; previous treatment, especially with insulin; introductory and maintenance doses of the drug; and the serum level of the drug. The results of the selection tests have been compared also with the results of the treatment.

The analysis has not taken into account the differences in reaction between youthful diabetics and middle-aged obese diabetics; these differences are well known, but in practice it is difficult to classify many patients according to rigid categories without doing violence to the facts of the case. In the present analysis greater emphasis has been given to the nutritional state

of the patient, a fact that can be determined readily. Results of treatment are presented in TABLE 9.

In practice, according to the criterion adopted, patients in whom we observed either a good or a fair response were considered subjects for long-term treatment, with this reservation: the fair cases must be subject to rigid observation, since it is possible for such cases to develop into poor cases as time passes, even when such development is not due to other causes such, for example, as dietary digressions. With this reservation it is obvious that approximately 80 per cent of the patients were susceptible to treatment with chlorpropamide.

Age as Related to Response

Treatment failed in the 2 patients under 20 years of age. In the 21 to 40 year group and the group over 40, the percentages of good cases are equal,

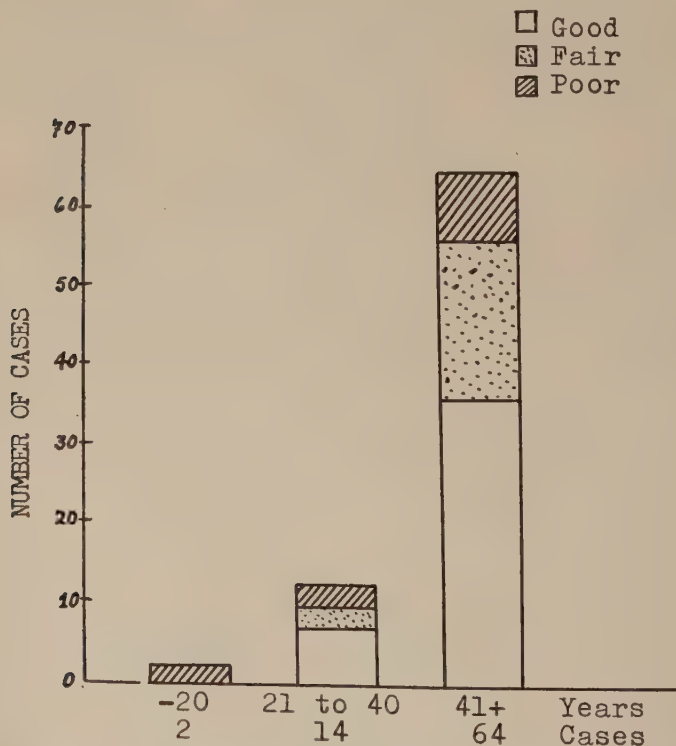


FIGURE 2. Age and results of chlorpropamide treatment in 80 patients.

but the failures are more frequent in the younger patients (21.4 and 12.5 per cent, respectively). This tendency reveals a definite correlation of age to response, but one that probably would have been more striking had the number of patients involved been greater (FIGURE 2).

Onset of Diabetes as Related to Result

This is a complex factor. Aside from considering the special characteristic of diabetes, this factor includes an unknown: the duration of the disease. Nevertheless, both factors suggest the same conclusions, that diabetes in younger patients is habitually refractory to oral treatment and, on the other hand, that there is a possibility that the disease existed for a longer period of time in those who develop diabetes while young (among our patients were 23 more than 40 years old in whom onset of the disease had occurred between their twenty-first and fortieth years). It was observed that when the onset of diabetes was between 21 and 40 years of age, the percentage of good results was 47.8 per cent; in those in whom the onset of the disease occurred after the fortieth year, good results were obtained in 60 per cent of the patients.

Duration of the Disease as Related to Response

The groups are rather small for a rigorous statistical analysis, but the influence of duration of diabetes seems to be considerable in patients treated with other hypoglycemic drugs.

Of those exhibiting a good result, 89 per cent were patients whose diabetes was of less than one year's duration. Of 22 patients who had had diabetes for more than 10 years, 12 responded well to chlorpropamide, while the remaining 10 showed a fair response. This result may be due to the interference of factors other than the duration of the disease. It may be probable that duration of the disease has little or no direct effect on the result obtained with treatment. It may be of importance in long-standing cases in an indirect manner, such as its influence on modifications of the nutritional state that frequently deteriorates in patients not receiving constant treatment.

Effect of Previous Treatment on Response

Of the 11 cases that had never been treated with insulin or other measures, the results in 10 cases were good (91 per cent) and, in 1 case, poor (FIGURE 3).

In patients who had received insulin, the results are inferior to the general average of results obtained, but the difference is not significant. Twelve of the 13 failures had received insulin previously, and 6 of them also had received treatment with sulfonylureas. However, this result should not be regarded as a direct consequence of the previous treatment factor exclusively, since 90 per cent of the thin, underweight patients had required insulin previously, but only 70 per cent of the obese patients had received it; and the influence that the nutritional state, constitution, and type of diabetes exert on the response to oral therapy is well known.

FIGURE 4 shows that the duration of insulin therapy constitutes an adverse factor on the response to treatment with chlorpropamide. In fact, 55 per cent of the patients who had used insulin for less than 5 years had good results with chlorpropamide and only 25 per cent of the patients who had used insulin for more than 5 years had such results. It should be noted that in the latter group more than half the patients initially had a fair response.

In regard to insulin dosage, even though the patients who receive high

dosage are few, we found that in the 7 patients studied who had received more than 40 U. daily, there was no case in which good results were observed with chlorpropamide therapy. We obtained 4 fair and 3 poor responses in spite of the fact that 4 patients were obese, 2 of normal weight, and only 1 was thin. This would confirm the fact that high insulin dosage is an adverse factor. The number of cases studied in our experiments is too small to permit an analysis of the combined effect resulting from the size of the

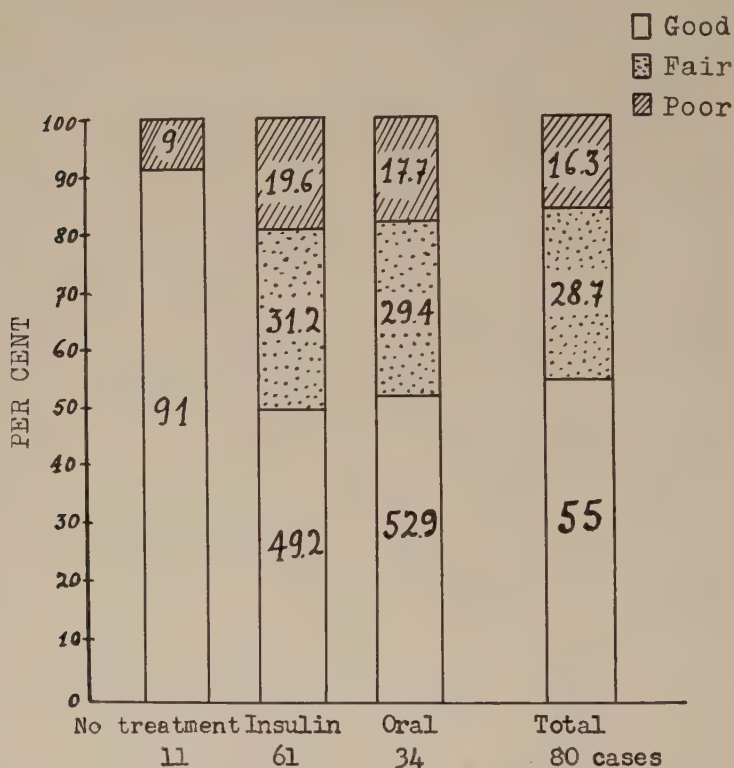


FIGURE 3. Prior therapy and results of chlorpropamide treatment in 80 patients.

insulin dosage received and the number of years insulin had been administered.

It is interesting to compare results of other types of oral therapy with the results obtained with chlorpropamide. TABLE 10 presents the results obtained using chlorpropamide and other orally applied agents (carbutamide and tolbutamide) on 29 patients. It may be observed that chlorpropamide brought about compensation in 9 of the 14 cases that were failures under the other drugs and achieved good results in 3 of the 6 cases that were merely fair under the other drugs. This is a fact of great therapeutic importance, since it offers the possibility of treating patients who have failed to respond to other forms of oral therapy. In TABLE 11 it may be seen that the over-all

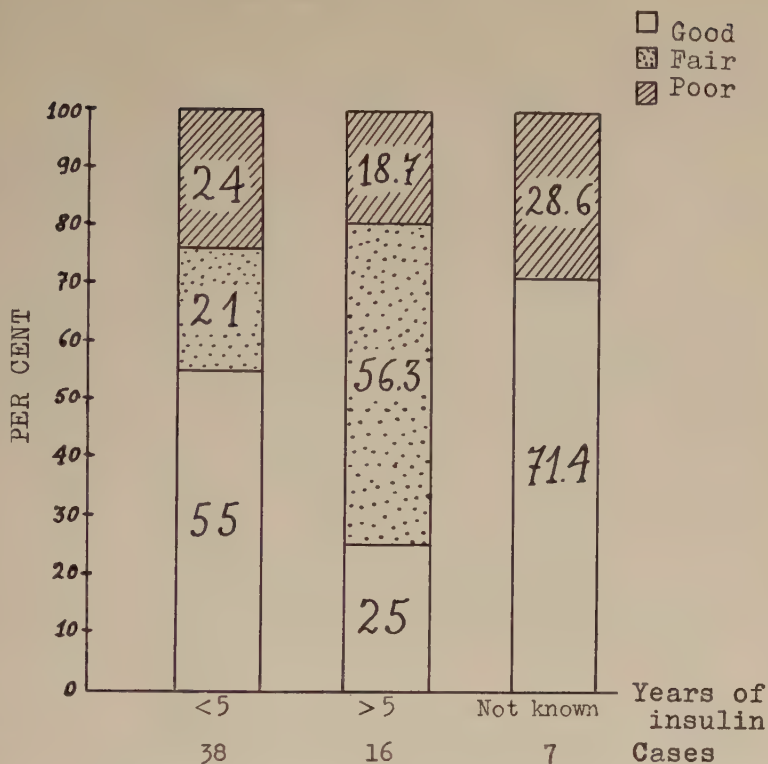


FIGURE 4. Years of insulin and results of chlorpropamide treatment in 61 patients.

TABLE 10
COMPARISON OF CHLORPROPAMIDE TREATMENT WITH OTHER HYPOGLYCEMIC AGENTS IN 29 CASES

Chlorpropamide	Other hypoglycemic agents			Chlorpropamide totals
	Good	Fair	Poor	
Good.....	8	3	5	16
Fair.....	1	2	4	7
Poor.....	—	1	5	6
Total.....	9	6	14	29

TABLE 11
RESULTS OF TREATMENT WITH 3 HYPOGLYCEMIC DRUGS

	Carbutamide		Tolbutamide		Chlorpropamide	
	No. cases	Percentage	No. cases	Percentage	No. cases	Percentage
Success*.....	169	84	56	72.8	67	83.7
Failure.....	32	16	21	27.2	13	16.2
Total.....	201	100	77	100.0	80	100.0

* Including good and fair results.

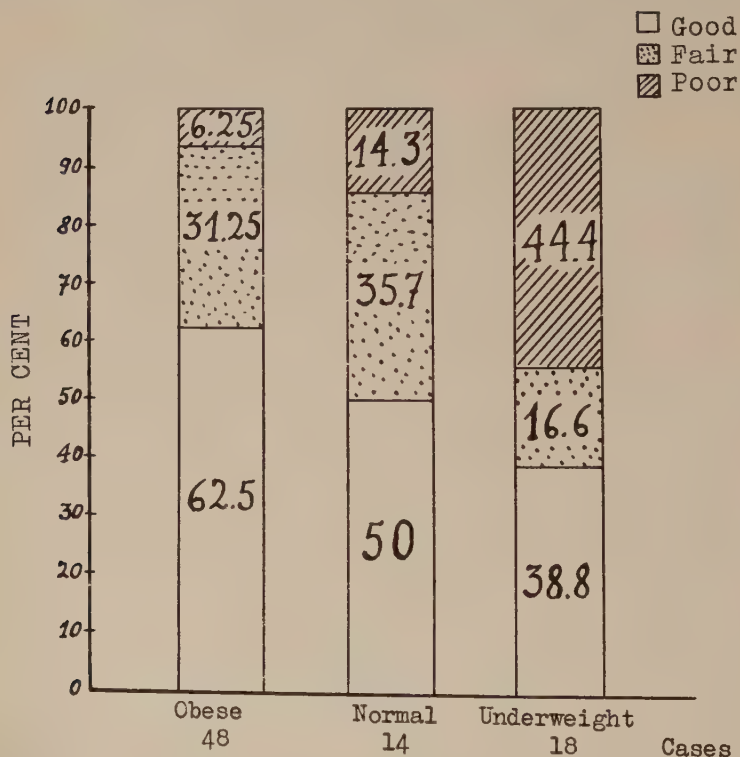


FIGURE 5. Results of chlorpropamide treatment and nutritive status in 80 patients.

experience of the authors is that chlorpropamide has demonstrated itself to be as effective as, or more effective than, carbutamide and tolbutamide in the control of diabetes.

Effect of Nutritional Condition on Response

From FIGURE 5 it is obvious that the highest percentage of success (62.5 per cent) was obtained in obese patients, just as is the case with the other sul-

fonylureas. Even in the group between 20 and 40 years of age, the percentage of success is high in these patients. It is not within the scope of this paper to discuss to what extent many of these patients may have been treated with a rigorous low-calory diet to provide compensation and reduce obesity. As a matter of fact in most of these patients such a procedure was practically impossible, since they were generally already receiving a moderate low-calory diet to conform with their activity, and they still required medication to control their blood sugar levels.

Effect of Complications or Intercurrent Disorders on Response

In general, the presence of retinopathy, nephropathia, and tuberculosis or other conditions did not affect the response to an appreciable extent as compared with patients who had no pathology other than diabetes mellitus. Clinically these complications were not aggravated during the course of treatment; on the contrary, good results were obtained in the tuberculous diabetic with chlorpropamide as with the other sulfonylureas. It should be mentioned that 7 of the 9 tuberculous patients benefited from chlorpropamide.

Effect of Dosage on Response

There was no apparent difference in response to an initial dosage of either 2 gm. or 1 gm.; the latter dose obviated, in part, the appearance of secondary

TABLE 12
MAINTENANCE DOSE AND RESULTS OF CHLORPROPAMIDE TREATMENT IN 67 PATIENTS

Result	Maintenance dose in mg.					Total
	125	250	500	1000	1500	
Good.....	2	12	15	13	2	44
Fair.....	—	—	3	16	4	23
Total.....	2	12	18	29	6	67
Percentage of Cases.....	3	18	27	43	9	

reactions, especially of a gastrointestinal digestive nature. TABLE 12 indicates the maintenance doses employed, grouping the patients according to the results. It should be noted that among the 13 failures high doses (2 gm. daily) were used in 7 cases, 1.5 gm. doses in 5 cases, and 1 gm. in only 1 case. These were considered failures only after 10 or more days of treatment with these dosages, thus ensuring that therapeutic blood levels of the drug had been reached. Nevertheless, despite the high dosages that often produced intolerance, as with the other sulfonylureas, poor responses were obtained only in those patients who were not susceptible to oral therapy for various reasons.

Response as Related to Observation Time

Of the 80 patients studied, treatment was continued in 67 (the 13 failures having been excluded). Of the 67, 62 underwent 30 or more days of treatment.

We record a fact known from treatment with the other sulfonylureas: in some cases, the results change from good to fair or poor as time passes, generally within several months. Such patients represent the so-called secondary failures. If one eliminates the cases in which decompensation is caused by persistent violations of the prescribed diet or by intercurrent disease, one is invariably left with cases for the course of which an explanation is difficult. With chlorpropamide, in 15 patients whose treatment was continued from 90 to 210 days, we did not observe this secondary-failure phenomenon; however, our observation period is still relatively short.

Response as Related to Serum Level of the Drug

From TABLE 13 it may be seen that the serum level of the drug varied from 6.1 to 12 mg. per cent in 26 of 34 cases that responded to the drug,

TABLE 13
SERUM LEVELS OF CHLORPROPAMIDE IN PATIENTS RESPONSIVE TO THIS DRUG;
DETERMINATIONS IN 34 PATIENTS

Serum levels of chlorpropamide	Good			Fair			Total
	250	500	1000	500	1000	1500	
3.1-6	—	2	—	—	1	—	4
6.1-9	1	5	3	2	1	1	13
9.1-12	1	2	2	—	7	1	13
12.1-15	—	—	2	—	—	1	3
21.1-24	—	—	—	1	—	—	1
Total.....	2	9	8	3	9	3	34

without any apparent indication that this serum level was related to the response to treatment. Two cases receiving 250 mg. of chlorpropamide daily had blood levels that did not differ from those obtained with higher dosage so that this small maintenance dose appeared to be as effective as the higher dosage.

Comparison of Clinical Results with Earlier Selection Tests

Response to treatment with sulfonylureas may be predicted, to a degree, by studying the hypoglycemic response obtained with a single dose of the drug. This selection test is not of much practical value in patients in whom there is a clear clinical indication that the drug would be effective, but its use provides some measure of guidance in relation to difficult cases.

It can be seen from TABLE 14 that of the 40 selection tests in which good results were obtained, only 3 had a poor response to chlorpropamide. However, it was also observed that in 21 patients who showed a poor response to the selection test, only 5 actually showed a poor response to treatment, which renders the test somewhat valueless when it is compared with the clinical indication.

Of greater interest is the test with regard to thin, malnourished patients. It may be observed that in this group the result of the test coincides very

TABLE 14
SELECTION TEST, NUTRITIVE STATUS, AND RESULTS OF CHLORPROPAMIDE
TREATMENT IN 67 PATIENTS

Selection test	Obese			Normal			Under weight			Total
	Good	Fair	Poor	Good	Fair	Poor	Good	Fair	Poor	
Good.....	15	6	2	4	3	1	7	2	—	40
Fair.....	2	2	—	—	—	—	—	1	1	6
Poor.....	7	7	1	—	1	—	—	1	4	21
Total.....	42			9			16			67

well with the response to treatment. The 9 cases with good tests all were susceptible to treatment; of the 5 tests with poor results, in only 1 case was the result of the treatment fair, the other 4 cases being failures. Our experience with thin patients indicates that the test is one that provides a rapid means of selecting those patients susceptible to oral treatment with chlorpropamide.

Side Reactions

Side reactions were observed in 24 patients. One third of these patients presented 1 symptom only, one third presented 2 or 3 symptoms simultaneously, and the last third, 4 or more symptoms.

Cutaneous reactions (pruritus and erythema with pruritus) were observed in 3 cases. We observed digestive manifestations in 20 cases (epigastric distress, nausea, vomiting, diarrhea, anorexia), and general symptoms in 15 cases (headache, asthenia, drowsiness, dizziness or vertigo, and muscular pains). The most frequent symptoms were epigastric distress (16 cases) and asthenia (14 cases). The frequency of the other symptoms varied between 2 and 7 cases.

Cutaneous manifestations were mild. In 1 case they disappeared spontaneously without interruption of therapy and in the other 2 cases they disappeared after the treatment was temporarily suspended for 2 days. In the 20 patients experiencing digestive manifestations, these reactions were mild in 50 per cent, moderate in 25 per cent, and severe in 25 per cent of the cases.

In the 15 patients who exhibited general symptoms, these were mild in 60 per cent, moderate in 15 per cent, and intense in 25 per cent of the cases.

Side reactions usually appeared between the third and fifth days of treatment (range 1 to 11 days), and lasted 4 to 5 days (range 2 to 14 days). They generally appeared when the patient had received a total of between 5 and 10 gm. of the drug (range 1 to 16).

With daily doses of 2 gm. we observed reactions in 38.4 per cent of the cases; with 1 gm., in 21 per cent. With a dose of one-half gram only, we observed 1 case of pruritus (which disappeared spontaneously) and no other reaction. The reactions were twice as frequent in the patients who responded poorly to treatment. This is understandable, since these patients received much higher doses for a longer period of time in an attempt to control the diabetes. Six of the 13 failures accounted for 31 per cent of the secondary reactions observed.

Since more than 70 per cent of the side reactions were mild or moderate, and since two thirds of those affected presented 1 to 3 symptoms, it was not necessary to suspend treatment in the majority of the cases. The symptoms disappeared spontaneously or, when the dosage was reduced, in 25 per cent of the cases; in only 6 cases was a temporary suspension of treatment necessary, usually for only 2 to 3 days. The treatment was discontinued completely in only 2 cases and, in both of these patients, intolerance to the drug coincided with therapeutic failure. To some patients we administered the drug in wafer form, which suggested (to the patient) a change of medication. This was followed by the rapid disappearance of epigastric distress in patients previously reporting this symptom. This occurrence suggested to us the presence of a psychological component that may have been responsible for the occurrence, intensity, and prolongation of side reactions, especially in neuropathic patients who felt that they were being subjected to experimentation.

Ten patients experienced an intense reaction as a result of ingesting alcohol during the treatment. In 2 cases this was produced deliberately in order to make clinical observations. This reaction has been described, and we have observed it since 1956 with other sulfonylureas. It takes the form of a reddening of the face, feeling of anguish, dyspnea, palpitations and, more rarely, vomiting; in 1 case the reaction was extremely violent and prolonged. This reproduces with absolute fidelity the reaction to tetraethylthiuram bisulfide (Antabuse), and can be interpreted as a consequence of blocking the oxidation of alcohol in the acetaldehyde phase.

It is therefore necessary to prevent the patient from consuming any alcohol, even in food. We should note at this point that there were patients who took very moderate amounts of alcohol (wine) at meals without sustaining any reaction, or only a mild one (reddening of the face exclusively); in other patients, after a greater or lesser period of treatment, the reaction did not recur, so they continued to use a small quantity of wine.

One female patient complained of hypoglycemic symptoms, without laboratory confirmation since they occurred while the patient was at home. On two occasions we noted moderate hypoglycemia in other patients. One of these was a young tuberculous patient, thin, with a diet providing 250 gm. of

carbohydrates. He began treatment in intense decompensation which has been controlled exclusively by dietary means.

Finally, one patient without alcoholic, hepatic, or biliary antecedents showed icterus of the cholangiolithic type after 18 days of treatment. Since this case is of especial interest, we reproduce the case history:

Patient, aged 44, male, diabetic since 1952, controlled in a very irregular way with protamine zinc insulin and diet; no vesicular or hepatic antecedents. On April 1, 1958, treatment was initiated with 2 gm. daily of chlorpropamide, which brought about compensation of the diabetes without any sign of intolerance or side reaction. The patient was discharged on April 11, 1958 on a maintenance dose of 250 mg. twice a day. Two days later discrete epigastric

TABLE 15
TESTS

Tests	3/27/58	4/19/58	4/23/58	5/2/58	5/22/58
Chlorpropamide dose.....	(2 gm. daily, 4/1/58 reduced to 250 mg.; twice daily 4/11/58, continued to 4/18/58)				
Bilirubin direct 1 min.....	0.3	1.8	1.8	0.2	0.3
Total.....	0.5	3.3	3.4	0.5	1.0
Total cholesterol.....	208	no	434	220	—
Alkaline phosphatase.....	3.2	10.7	10.7	10.7	3.2
(Hanger) flocculation test.....	(—)	(—)	(—)	(—)	(—)
Prothrombinemia.....	100%	no	100%	no	—
Bromsulphalein test*.....	7.6%	no	no	no	0%
Mucoproteins.....	no	no	4.6	no	no

* Normal: retention less than 6 per cent at 45 min.

pain without radiation and with no correlation to ingestion of food, also anorexia, dark urine, and discolored stool were noted. On April 15, 1958 conjunctival and cutaneous jaundice appeared associated with moderate pruritus. Prior to treatment with chlorpropamide the patient had shown normal results in the following tests: urine examination, hemogram, sedimentation test, bilirubin determination, alkaline phosphatase test, flocculation test, Bromsulphalein determination, creatinine clearance, and phenol-sulfonphthalein test. He was rehospitalized on April 18, 1958, and chlorpropamide was discontinued.

On physical examination the patient was conscious and lucid; pulse 70; regular, arterial pressure 110/70; no fever; good general condition; moderate icterus of skin and mucosa; hepatomegaly of two-fingers' breadth under rib line; spleen not palpable. Other examinations were normal.

The liver went down to normal size two days after admission, and the icterus disappeared clinically 17 days after having first appeared; the patient was discharged on May 8, 1958, compensated with 32 U. of protamine zinc insulin daily. Colecystography 2 months after discontinuation of chlor-

propamide treatment was normal, and the patient has remained in good health up to his last examination (August 23, 1958): flocculation tests, negative; bilirubin determinations, normal; and Bromsulphalein retention at 45 minutes, 3.6 per cent (normal, less than 6 per cent). We were not able totally to exclude the possibility of a hepatitis of virus origin, frequent in our environment, but from the time of appearance, the cholangiolitic character, the brief duration, and the subsequent course, we are inclined to interpret the case as an icterus of toxic origin (TABLE 15).

Laboratory Determinations

Hanger reaction. The hanger reaction was determined in 23 patients. In 21 it was negative and remained so throughout tests made from 20 to 210 days after beginning treatment. In 2 cases it was positive and persisted from the beginning for 27 and 28 days, respectively. Suspension of treatment was considered for these two patients.

Alkaline phosphatase. This enzyme was studied in 21 patients. In 18, it remained normal between 30 and 210 days of treatment; in 2 cases it increased moderately, but became normal at 20 and 28 days, respectively; and it increased considerably in 1 case (cholangiolitic icterus).

Bromsulphalein retention. Thirty-seven patients were studied in this connection. In 27 cases the results were normal (less than 6 per cent) between days 30 and 210 of treatment. In 3 cases, in which the percentages were moderately increased before initiation of treatment, values became normal within 52 and 70 days. Three cases in which Bromsulphalein retention had been increased did not change during a course of treatment lasting 56 to 120 days. In 3 cases that were normal before therapy, very moderate increases occurred in 65 to 107 days (8.3 to 7.8 to 10.4 per cent).

Prothrombin. This factor was studied in 27 cases. In 25 cases, it remained normal between 30 and 210 days. In 1 case there was a moderate decrease at 84 days (without alterations in other tests). In 1 case there was a decrease that lasted for 7 days before treatment was suspended because of an intervening illness.

Hemogram. The blood picture was studied in 47 cases. In 3 cases we observed moderate but insignificant decreases in the erythrocytes between 30 and 105 days of treatment. In 1 case there was a very moderate decrease in leukocytes (4200) after 60 days of treatment.

Urine. Alterations were observed in only one case, where albuminuria (0.64 gm. per cent) appeared after 80 days. The interpretation of this case is difficult. In the 5 cases in which albuminuria had existed previously, there was no modification up to 30 to 120 days of treatment.

DISCUSSION

The most significant facts encountered in this clinical test of chlorpropamide are the evidence of greater activity of this new sulfonylurea and the increased frequency of secondary reactions when the minimal effective dose is exceeded. Other aspects of clinical response are the same as those observed with the use of similar substances, as may be deduced from analysis of data

in relation to age, nutritional status, and the other variable considerations always taken into account in the oral treatment of diabetes mellitus.

The greater activity of chlorpropamide is principally a consequence of the fact that it rapidly reaches effective levels in the blood, probably a result of its being excreted slowly. This also explains the general symptoms observed when doses higher than necessary are used, as found with the first patients we treated. The digestive symptoms, on the other hand, especially the epigastric distress, can be attributed to a definite direct irritating action that probably comes from the presence of chlorine in the molecule. It should be noted that most of these manifestations are only of mild or moderate intensity and of short duration; they usually disappear even with continuation of therapy. From a practical viewpoint, these manifestations do not constitute obstacles to the clinical use of the drug except for an occasional patient who, in our experience, usually is also a therapeutic failure.

Examinations of blood and urine and tests of hepatic function revealed no significant modifications in observation periods lasting up to 210 days. The only serious complication might be represented by a case of hepatitis that rapidly ran its course; since that time no sequelae have become obvious.

Like the other sulfonylureas, chlorpropamide is active within a few hours after initial administration, but there appears to be a period of latency before the blood sugar begins to decrease, during which effective levels of the drug develop in the blood. Another noteworthy fact is the reaction observed when alcohol is ingested during therapy. Apart from the practical interest and importance such a fact has in areas where the consumption of a moderate and permissible amount of alcohol is habitual with meals, there is a theoretical consideration in that it reveals another aspect of the action of the sulfonylureas, one by which they directly modify the intermediate metabolism, acting on the enzymatic processes.

Finally, we are not able to exclude from the observed successful responses a certain factor of suggestibility that may have resulted in a better compliance on the part of patients to dietary instructions. Nevertheless, this is a factor that has not yet been eliminated in any published studies and that could serve only to vary the absolute figures for successful results to a minor degree, at the same time still permitting comparisons to be made among various drugs.

SUMMARY

Chlorpropamide was administered to 80 diabetics (38 males and 42 females). Two of these patients were less than 20 years old, and 64 were older than 40 years; 48 were obese, 14 of normal weight, and 18 underweight. The diet contained 150 to 250 gm.; maintenance dosage ranged from 125 mg. to 1.0 gm. Patients were observed from 30 to 210 days. With the initial dose, the immediate hypoglycemic effect was studied on a fasting state. The greatest decrease in the blood sugar level took place at 5 hours in cases that responded (selection test).

Disappearance of glycosuria and lowered blood sugar levels were obtained in 58 per cent of the cases in the first week and in 31 per cent in the second

week. Results of treatment were good in 55 per cent, fair in 29 per cent, and poor in 16 per cent. In the group exhibiting good results, 75 per cent were observed for more than 60 days; 66 per cent of the cases were maintained on a dosage of 500 mg. or less (plasma chlorpropamide ranged between 4.6 and 9.8 mg./100 ml.).

Results were correlated directly with nutritional status, age of the patient, age at onset of the diabetes, and absence of previous treatment. An inverse correlation was found to duration of treatment and dosage of insulin. No correlation was found to duration of diabetes mellitus.

There were secondary reactions in 24 cases including skin (3), digestive (20), and general manifestations (15). Such symptoms were minor in 72 per cent and disappeared spontaneously after a decrease in dosage or after a short period of discontinuation (6 cases). With a dosage of 1 gm., 21 per cent of reactions were observed as compared with 38.4 per cent when 2 gm. was given. One of the patients experienced jaundice of cholangiolitic type after 18 days of therapy. Treatment was discontinued. Alcohol ingestion induced anxiety, palpitations, and flushes in 10 cases. No changes were found in liver, kidney, or blood forming functions during or after treatment.

ACKNOWLEDGMENTS

We thank M. Usano of Chas. Pfizer & Co., Inc., Brooklyn, N. Y. and Laboratorios Pfizer de Chile, Santiago, Chile for making available the chlorpropamide (Diabinese) used in this study, and for technical data used in determining the presence of chlorpropamide in serum.

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CLINICAL STUDY OF THE EFFECTS OF CHLORPROPAMIDE ON NORMAL SUBJECTS AND ON DIABETICS

Luis J. Cardonnet, Juan J. Staffieri, Derio R. Eberhardt,
Pedro O. Tommasino, Ricardo R. Berli, Jorge Muratorio

Institute of Endocrinology and Nutrition, British Sanatorium, Rosario, Argentina

Since the year 1955, when Franke and Fuchs published a paper in which they recommended the use of the sulfonylurea carbutamide (1-sulfanilyl-3-butylurea, or BZ 55) for the oral treatment of diabetes mellitus, there have been numerous articles describing clinical experiments conducted with other compounds including *p*-aminobenzenesulfamidyl-*tert*.-butyl (RP 2259); and tolbutamide (1-butyl-3-*p*-tolylsulfonylurea or D 860). At present tolbutamide appears to be preferred by many researchers.

In the hope of finding a product that might be useful to all diabetics and have effective hypoglycemic action and a very low index of toxicity, research has continued in that direction; lately there have been tested some derivatives of guanidine, especially phenethylformamidinyliminurea (DBI) and, very recently, a new sulfonylurea, 1-propyl-3-(*p*-chlorobenzenesulfonyl)urea (P 607) commonly referred to as chlorpropamide.

The purpose of this article is to review the results achieved through the use of chlorpropamide (P 607) in persons who showed no disorders of carbohydrate metabolism and in those with diabetes. It was administered to both groups for evaluation of its hypoglycemic activity and for determination of its possible untoward or toxic effects and its therapeutic usefulness.

PLAN OF STUDY

Material and Methods

Thirty hospitalized patients who evidenced no disorders of carbohydrate metabolism and 60 patients with diabetes mellitus from our private practice,

TABLE 1
PRIVATE PATIENTS

Total patients studied, 60: outpatients 56, inpatients 4.

Sex: male 29, female 31.

Age: average 51 (range 6 to 76).

Duration of diabetes: recent to 36 years.

Type of diabetes: stable, 51; unstable, 9.

Days of treatment: variable (15 to 90 days).

Initially with diet alone: 6.

Initially with other hypoglycemics: 20.

Initially with insulin: 34.

Patients in more than one group: 32.

Subjects of hormone studies: 27.

the data on whom are compiled in TABLE 1, were selected for this research, which covered a period of 90 days. In the course of this work the following studies were made.

Studies in Nondiabetic Subjects Without Disorders of Carbohydrate Metabolism

With a view to determining the absorption and elimination curve of the compound and the ratio between the blood concentration reached and the hypoglycemic effect obtained, the subjects of this study were 30 inpatients (10 males and 20 females) who evidenced no metabolic disorder with respect to carbohydrates and who were divided into 3 similar groups of 10 patients each.

After prior stabilization on a prefixed diet (total caloric intake 1833.5 cal.: carbohydrates 35 per cent, 650 cal., 162.5 gm.; proteins 21 per cent, 396 cal., 99 gm.; fats 44 per cent, 787.5 cal., 87.5 gm.), the subjects were respectively administered a single dose of 0.5 or 1.5 gm. chlorpropamide. In all of the patients the blood glucose concentration was determined and, in 20 of them, that of chlorpropamide in the fasting state and 4 hours later, in the manner described by Camerini Dávalos *et al.* (1957). The same determinations were repeated in 2 subjects from each group after 8, 12, 48, and 96 hours.

Studies of Diabetics

Studies were made of 60 patients classified in 9 different groups with a view to investigating various aspects of the problem; most of them were studied from more than one aspect, and therefore appear in more than one group. This accounts for the apparent disparity between the actual number of patients studied and the number resulting from totaling the members of the different groups.

During the research a quantitative diet was indicated for all of the patients, the general characteristics of which were as follows: 150 to 200 gm. of carbohydrate and 75 to 105 gm. of protein, supplemented by fats in sufficient quantities to attain the total caloric intake desired for each particular patient.

Described below is the series of 5 biochemical determinations made, in addition to the findings of complete clinical examinations conducted, with a view to ascertaining the evolution of diabetes and verifying at an early stage any disorder that might occur in the hematopoietic system or in hepatic and renal function: (1) glycemia by Folin and Wu's serum method, modified for the determination of true glucose; (2) uremia by Sagastume and Oliva's gasometric method; (3) complete examination of urine; (4) peripheral hemogram; and (5) hepatogram, including Hanger's test, thymol turbidity, Kunkel's test, alkaline phosphatase, cholesterolemia, bilirubinemia, fractional proteinemia, and electrophoresis on paper.

Group 1. This group was composed of 10 patients who initially received a dosage of chlorpropamide for 48 hours of 1.5 gm./day, divided into 2 or more doses, followed by 1 gm./day for not less than 15 days. For the purpose of verifying the occurrence of untoward symptoms and gastrointestinal tolerance these patients also received, at various times, and under different circumstances, antacids, proportioning of doses, and administration concurrently with ingestion of food.

In 5 patients the drug was beneficial, and dosage was reduced to a minimum

effective level. These patients were added to the group of diabetics receiving protracted treatment.

Group 2. This group included 20 diabetics who were well balanced with other hypoglycemic compounds; 8 received carbutamide, and 12 received tolbutamide (TABLE 2). The initial dose of chlorpropamide, which was started immediately after suspension of previous therapy, was 1 gm./day for 2 days, and we attempted to reduce it rapidly to lower levels, according to the therapeutic response obtained. The purpose of this procedure was to make a comparative clinical study of various hypoglycemics in relation to such factors as the efficacy of a dose administered for a protracted time,

TABLE 2

Age and sex (present age; females are indicated in parentheses):

Ages:	1 to 10	11 to 20	21 to 30	31 to 40	41 to 50	51 to 60	61 to 70	71 to 80
	—	—	—	—	2 (1)	9 (4)	8 (5)	1 (1)

Age at onset of diabetes:

Ages:	1 to 10	11 to 20	21 to 30	31 to 40	41 to 50	51 to 60	61 to 70	71 to 80
	—	—	—	2	8	9	1	—

Years of diabetes at start of treatment:

Years:	Recent to 1	1 to 2	3 to 5	6 to 10	11 to 15	16 to 20	21 to 36
	—	4	5	1	6	3	1

Body type: pyknic, 1; normal, 18; longilineal, 1.

Type of diabetes: stable, 20 (100%); unstable, 0.

Prior drug therapy:

D 860	0.5	1 gm.	1.5 gm.
BZ 55	—	9	3
	4	4	—

Duration of prior treatment:

Less than 6 months	6 months to 1 year	1 to 2 years
4	8	8

Prior control: good, 19; fair, 1.

tolerance, and the continuance of proper regulation after suspension of the drug.

In all of these patients chlorpropamide therapy was continued for a prolonged period. Five patients from group 1 and 16 from group 4, who also continued to receive the drug, joined them, thereby raising to 41 the total number of subjects in whom the effects of the compound administered for a longer period in useful therapeutic doses could be observed.

The criteria applied in evaluating the metabolic equilibrium attained were as follows: *Excellent*, good clinical state, normoglycemia and aglycosuria for 24 hours; *Good*, good clinical state, glycemia at fasting level lower than 150 mg. per cent, and glycosuric discharges no greater than 10 gm. in 24 hours; *Fair*, Good clinical state, glycemia at fasting level up to 180 mg. per cent, glycosuric discharges less than 20 gm. in 24 hours; and *Poor*, cases not meeting the above-mentioned conditions.

Group 3. This group comprised 9 patients in whom carbutamide and tolbutamide had been inefficacious. These patients had received insulin and were selected with a view to drawing clinical conclusions on the similarities between the different compounds. The group includes one case that had presented a good response, but that was complicated by skin eruption and icterus.

Group 4. In this group were 34 cases who had never before been treated with oral hypoglycemics, in 8 of whom no improvement had been produced by dietary restriction, and who had required insulin for years for the proper regulation of their diabetes. The data on the members of this group are set forth in TABLE 3.

When insulin requirements had been lower than 30 U., insulin therapy was suspended, and treatment with an average dose of 1 gm. of chlorpropamide was started immediately. On the other hand, in those patients who had received more than 30 U., along with the beginning of treatment with hypoglycemics (125 to 250 mg./day), the dose of which was then gradually augmented, the dosage of insulin was scaled down by a percentage equivalent to 20 per cent of the previous dose every 3 or 4 days.

TABLE 3

Age and sex (present age; females are indicated in parentheses):

Ages:	1 to 10	11 to 20	21 to 30	31 to 40	41 to 50	51 to 60	61 to 70	71 to 80
	3 (3)	3 (1)	1	2 (1)	5 (1)	10 (6)	8 (6)	2 (2)

Age at onset of diabetes:

Ages:	1 to 10	11 to 20	21 to 30	31 to 40	41 to 50	51 to 60	61 to 70	71 to 80
	5	1	3	4	8	10	2	1

Years of diabetes at start of treatment:

Years:	Recent to 1	1 to 2	3 to 5	6 to 10	11 to 15	16 to 20	21 to 36
	1	5	12	10	2	3	1

Body type: pyknic, 2; normal, 28; longilineal, 4.

Type of diabetes: stable, 25 (75 per cent); unstable, 9 (25 per cent).

Prior dose: 30 U. or less More than 30 U.

18

16

Duration of prior treatment:

Less than 1 year	1 to 5	6 to 10	11 to 20	More than 20 years
—	7	11	12	4

Prior control: good, 24; fair, 10; poor —.

Group 5. In this group were 6 patients who were well regulated by dietary restriction alone. When under therapy, these patients were permitted a free and ample diet or prescribed 300 gm. of carbohydrates daily for the purpose of observing to what extent a sustained dose of chlorpropamide ranging from 125 to 500 mg. would make possible an ad libitum diet. They were administered the compound for periods of 30 days or more.

Group 6. This included 2 infant diabetics and 3 juvenile unstable diabetics, in whom chlorpropamide was tested in doses ranging from 125 mg. to 1.5 gm.; at the same time we attempted to reduce the insulin dosage to a minimum.

Group 7. This involved 17 diabetics in whom the effect exerted on thyroid function by the administration of other hypoglycemic compounds had already been studied. By means of the determination of serum protein-bound iodine (Barker and Humphrey's method), of basal metabolism, and of I^{131} uptake observed on the gland after 2 and 24 hours, the possible influence that might be exerted by chlorpropamide, when administered over a protracted period in an average dosage of 1 gm. daily, was studied.

Group 8. The following determinations were made in 10 diabetics, with a view to checking any disorder that might occur in hypophyseal and supra-

renal endocrine function: hypophyseal gonadotropins by Albright, Reifenshein, and Griswold's method; urinary neutral 17-ketosteroid by Callow, Gallow and Emmen's method; and urinary 17-hydroxycorticoids by Reddy, Renkins, and Thorn's method.

Group 9. This was a heterogeneous group of 8 diabetic patients who manifested the special conditions or associated endocrinopathies listed below.

These patients were administered chlorpropamide in varying dosages for the purpose of observing the changes that might occur in the existing equilibrium with respect to the carbohydrate metabolism: insulin resistance, 1; allergy to insulin, 3; adrenogenital syndrome through hyperfunctioning adenoma, 1; hyperthyroidism (postirradiation through exophthalmic goiter) treated with thyroids, 1; hyperthyroidism in evolution, 1; and leprosy, 1.

In the last 4 groups control determinations were made whenever it was possible to establish a minimum effective dosage gradually reached after initial doses of 250 to 500 mg.

RESULTS AND THEIR INTERPRETATION

Studies of Nondiabetic Subjects

The results obtained in this study are tabulated in TABLES 4 and 5, in which have been compiled all of the blood glucose concentrations and chlorpropamide values, expressed in milligrams per 100 ml. of blood.

From study of the values transcribed it can be concluded that chlorpropamide, when administered orally in humans, is rapidly absorbed and reaches efficacious blood concentrations in a few hours. The level attained, as far as the doses used by us are concerned, remained in direct ratio to the quantity administered and was sustained with proportionally the same differences during the first 48 hours. After 96 hours there was a marked decline in the values which, in 4 of 5 cases observed, failed to show any significant differences from one another. The decline registered in the glycemic values was quite pronounced within 4 hours after ingestion, since the chlorpropamide concentration had by that time reached at least 2.5 to 3.5 mg./100 ml.; we believe these concentrations are the minimum levels for therapeutic efficacy. The hypoglycemic action remained evident for the first 24 hours, diminishing during the following hours, despite the fact that there were effective blood levels, a fact we ascribe to the alimentary ingestion after the first day of observation.

It must be emphasized that in this experiment the glycemic decline was always pronounced, but did not remain in direct proportion to the blood concentration attained by the drug, since the latter had exceeded effective blood levels (greater than 3.5 mg./100 ml.).

Studies of Diabetics

Group 1. Seven patients of 10 received high doses for several days and 12 took a high dose only initially. Aside from the hypoglycemic effects produced in these patients, untoward side effects were manifested (TABLE 6) and, on various occasions, some of them experienced more than one reaction.

TABLE 4

Dose of chlor-prop-amide	Case No.	Glucose mg./100 ml.		P 607* mg./100 ml. 4 hr.	Dose of chlor-prop-amide	Case No.	Glucose mg./100 ml.		P 607 mg./100 ml. 4 hr.	Dose of chlor-prop-amide	Case No.	Glucose mg./100 ml.		P 607 mg./100 ml. 4 hr.
		Fasting	4 hr.				Fasting	4 hr.				Fasting	4 hr.	
0.5 Gm.	1	94	73	—	1 Gm.	11	92	68	—	1.5 Gm.	21	100	70	10.8
	2	78	56	2.8		12	83	62	7.7		22	89	80	—
	3	100	84	1.5		13	83	64	8.4		23	87	77	10.4
	4	92	61	—		14	121	78	—		24	94	72	—
	5	118	69	2.5		15	75	67	—		25	90	60	11
	6	90	70	4.6		16	113	70	10.2		26	120	90	9.4
	7	90	75	—		17	97	—	8.8		27	91	68	10.4
	8	97	68	1.5		18	76	74	8.2		28	77	57	9.6
	9	91	77	—		19	96	72	8.6		29	95	62	10.6
	10	82	69	—		20	103	75	6.7		30	74	55	9.2

* P 607 = chlorpropamide.

TABLE 5

Dose of chlorpropamide	Case No.	mg./100 ml.	Fasting	4 hr.	8 hr.	12 hr.	24 hr.	48 hr.	96 hr.
0.5 Gm.	5	Glucose	118	69	60	70	65	93	92
		Chlorpropamide	—	2.5	5.6	3.3	—	4.8	2.3
	6	Glucose	90	70	93	81	65	90	96
		Chlorpropamide	—	4.6	2.1	3.1	—	2.3	1.5
1 Gm.	17	Glucose	97	—	103	72	89	94	108
		Chlorpropamide	—	8.8	5.2	5.7	—	4.8	1.9
	18	Glucose	76	74	75	77	64	101	112
		Chlorpropamide	—	8.2	5.9	5.9	—	5.4	3
1.5 Gm.	21	Glucose	100	70	98	88	83	103	100
		Chlorpropamide	—	10.8	12.1	9.0	—	8.8	3.1
	23	Glucose	87	77	47	63	68	90	—
		Chlorpropamide	—	10.4	10.1	10.1	—	7.3	—

TABLE 6

TOTAL UNTOWARD SIDE EFFECTS OBSERVED

Facial flushing and feverishness: 9.

Skin reactions: 4.

Digestive disturbances (pyrosis, regurgitation, bloated feeling, anorexia): 10.

Constipation: occasional, impossible to determine accurately.

Headache: 3.

Feeling of general malaise, with vertigo: 2.

Increase in blood pressure?: 2.

It is to be pointed out that reactions of the vasomotor type accompanied by feverishness and facial flushing frequently broke out after ingestion of an alcoholic beverage, spontaneously receding within 30 min. and failing to occur when the dose of the drug was reduced or the ingestion of alcohol was suspended.

The skin reactions were largely minor eruptions and, on two occasions, became evident after the administration of a saline laxative. In two patients it was necessary to discontinue the drug, application of which could be resumed without side reaction only in smaller doses once the cutaneous episode had subsided.

Gastrointestinal disorders occurring largely in the upper digestive tract were the disturbances most persistently complained of by the patients.

These manifestations could be relieved with the administration of antacids and proportioning of dosage of the drug prescribed, and by the ingestion of both at meal times, but the disorders could not be totally eliminated until the dosage was reduced below 1 gm./day.

Some patients complained of constipation, occasional headache, and malaise and showed an increase in blood pressure; all of these disturbances were transitory, and their cause and effect relationship with chlorpropamide cannot be defined by the present study. Investigations of liver function and blood count were unsuccessful in relating any of the above-mentioned disturbances to a possible attack on the hepatic parenchyma or hematopoietic system. Analyses of blood and urine urea did not show any significant changes.

Five patients in this group subsequently continued therapy with doses of chlorpropamide not exceeding 750 mg./day.

Group 2. In none of the 20 patients who had been kept well regulated with the use of carbutamide or tolbutamide was any disturbance observed when the previous therapy was suspended and chlorpropamide immediately substituted. Chlorpropamide proved efficacious in all of these cases.

Six patients who had been using carbutamide were satisfactorily regulated with an average dose of chlorpropamide approximately equivalent to one half the dose of carbutamide received. In those patients who had received tolbutamide, the effective dosage of chlorpropamide was determined to be 3 times (in some instances 4 times) smaller than that of the hypoglycemic previously used.

Group 3. In 4 of 9 patients in whom other oral hypoglycemics had been ineffective, the same poor results were observed when chlorpropamide was used; in the other 5 cases chlorpropamide produced satisfactory regulation in contrast to the results previously obtained with carbutamide and tolbutamide. It should be pointed out that in 2 of the latter patients the failure of the drug previously used was due partly to untoward side effects that contraindicated their use (severe icterus). It should also be noted that on 2 occasions patients well balanced with chlorpropamide required insulin during the acute period of an intercurrent febrile process.

Group 4. In 8 cases receiving an insulin dose of less than 30 U., it was possible to suspend insulin completely and replace it with chlorpropamide. On the other hand, only 5 patients receiving more than 30 U. of insulin daily could forego the injections.

In 6 cases the insulin dosage, which had ranged between 40 and 70 U., could be significantly reduced (by 20 per cent or more) when chlorpropamide was added to the treatment.

Group 5. In all of the cases making up this group the addition of chlorpropamide to dietetic treatment made it possible to broaden the diet to normal limits without untoward effects. It must be pointed out, however, that repeated dietetic transgressions or excessive gain in weight contraindicated the continuation of this leniency in the majority of the patients.

Group 6. In none of the 5 members of the group was it possible to suspend insulin entirely; however, in 2 cases it was possible to reduce the dose by

50 per cent of the previous quantity when chlorpropamide was also administered in quantities of 1 and 1.5 gm./day, respectively. In a third case the reduction of the insulin dose was equivalent to only 10 per cent of the previous dose, but perfect metabolic equilibrium was obtained without hypoglycemia which until then had recurred daily.

Group 7. In none of the 17 patients making up this group were there any clinical manifestations attributable to hypothyroidism. The variations in values obtained after administration of the drug for long periods and in doses

TABLE 7
THYROID STUDY

	I ¹³¹ (percentages)		Serum protein-bound iodine (μ g.)	Basal metabolism (per cent)	Observations
	2 hr.	24 hr.			
1	27	47	3.3	+ 1	Hypothyroidism (under treatment)
2	11	4	1.9	+14	
3	25	47	3.3	+33	
4	20	44	4.1	- 3	
5	23	44	3.7	\pm 0	
6	17	36	2.6	+18	
7	20	53	3.6	+ 8	
8	19	31	4.1	-19	
9				+25	Hyperthyroidism
10				- 2	
11	41	62	9.1	+30	
12	33	44		+ 0	Hyperthyroidism
13	34	56	8.0		
14	10	34	3.1	+40	
15				-12	
16				- 9	
17	15	32	3.7	+ 5	

of not less than 1 gm. showed no significant difference, in the sense of functional inhibition of the thyroid gland, a circumstance that had been observed in 8 of these patients while they were receiving carbutamide. Without reaching statistically significant levels, serum protein-bound iodine showed a slight tendency to diminish in value (TABLE 7).

Group 8. The results of the hormonal determinations made in this group of patients and summed up in TABLE 8 do not make it possible to state that chlorpropamide exerts any influence on the glandular functions studied.

Group 9. Among the 8 patients with special conditions, in the patient who demonstrated resistance to insulin it was possible to reduce the insulin dose from 500 to 60 U./day when chlorpropamide (2 gm./day) was added to the treatment; this drug also contributed to greater metabolic stability

and relieved the patient of the recurrent periods of chemical acidosis from which he constantly suffered.

The 3 patients with allergy to insulin tolerated this new substance without incident. In the patient with the adrenogenital syndrome, in spite of the extraordinarily high content of urinary 17-ketosteroids (120 mg. in urine after 24 hours), the response to chlorpropamide was similar to that registered in the cases without endocrinological complications.

TABLE 8
URINARY DETERMINATION OF HYPOPHYSEAL GONADOTROPINS; NEUTRAL
17-KETOSTEROIDS AND 17-HYDROXYOXOSTEROIDS

Case No.	Gonadotropins		17 K.S. mg./ 24 hours	17 H.K.S. mg./ 24 hours	Observation
	To 6 U.L.	To 96 U.L.			
1	(+)	(-)	7.20	2.63	—
2	(+)	(-)	—	—	—
3	(+)*	(-)	6.44	1.68	—
4	(+)	(-)	—	—	—
5	(+)*	(-)	4.68	4.45	—
6	(+)	(-)	5.40	6.98	—
7	(+)	(+)*	1.20	2.19	Adrenogenital syn- drome
8	(+)	(+)	4.62	7.98	Menopause
9	(+)	(+)	8.30	3.41	Menopause
10	(+)	(-)	6.21	4.82	—

* Weak.

In both patients with functional disturbance of the thyroid gland it was extremely difficult to obtain adequate control, as had been the case with all of the treatments previously employed, because of the accentuated metabolic instability with marked ketosis trend constantly present.

In the leprous patient the response obtained with chlorpropamide was to be expected, taking into account solely his metabolic disorder.

COMMENTS

Chlorpropamide is a compound very similar in chemical structure to carbutamide and tolbutamide,



having in common with them the part included in the box drawn with broken lines, but differing in that it has a propyl radical instead of butyl, and that in the para position of the benzene nucleus it has a chlorine instead of the amino radical (NH₂) of carbutamide and the methyl (CH₃) of tolbutamide.

As indicated by Menhert and Menhert (1956) with respect to tolbutamide:

the absence of the amino radical in chlorpropamide means that it has no bacteriostatic action, for which reason undesirable changes in intestinal flora are not registered when it is used. This fact has been corroborated by this study. On the other hand, the aforementioned disorders of the upper gastrointestinal tract become common (in about 70 per cent of the subjects studied) when daily doses of more than 1 gm. are administered. In no case did we witness such effects when the dosage did not exceed 750 mg./day.

The curves pertaining to nondiabetics reveal the rapid absorption of the compound and its marked therapeutic efficacy in doses of 0.5 gm. and even less, since after 4 hours the glycemic levels had descended by at least 20 per cent with respect to the initial levels. The greater quantities resulted in a higher blood concentration of the drug and in its slower elimination from the blood stream (good concentrations after 96 hours), which accounts for the protracted therapeutic effect after suspension of the drug and for the high concentrations attained with repeated doses. These observations coincide with other experimental and clinical findings (Goldner, 1958) to the effect that effective blood levels, attained after 48 hours, slowly drop on suspension of the drug until its complete elimination from the blood stream within a period of 5 to 7 days. According to Heinsen (1958), 50 per cent of an oral dose will still remain in the blood after 24 hours. This opinion is shared by Miller (1958), according to whose experience, with a dose of 1 gm./day, the blood concentration rises gradually for a period of 1 week and is then maintained at the last levels reached.

In one of our cases in which the maintenance dose was 1.5 gm./day, 25 days after treatment the blood chlorpropamide concentration was 16.5 mg./100 ml.; in 3 other patients receiving 750 mg. over the same period, the values were 8.4, 10.8, and 13.3 mg./100 ml., respectively. In one of the patients who received 500 mg./day, the figure was 9.4 mg./ml.; on the other hand, in 2 other cases with the same dose the values were 4.6 mg. and 5.4 mg./100 ml., which reveals the importance of the size of the dose by itself and the variations that can arise according to the individual susceptibility of each patient.

Taking the foregoing into account and also the fact that the hypoglycemic effect bore no direct relationship to the dosage, since effective therapeutic levels had been attained, it does not seem hazardous to affirm that the quantities indicated for clinical use should not exceed 750 mg./day, whether in single or proportioned doses, the more so since the risks of hypoglycemia and the onset of untoward side effects become more common with higher doses.

Without being able to point to a definite pathogenic mechanism (perhaps the inhibition of alcoholic dehydrogenation), mention should be made of the frequency with which we observed a reduced alcoholic tolerance, manifested by feverishness and facial flushing, which ceased with the reduction of the dose or suspension of alcohol.

The possibility of satisfactorily regulating diabetics who had previously been receiving carbutamide or tolbutamide successfully was confirmed in 100 per cent of the cases studied in such connection, and it must be added that chlorpropamide was also efficacious in cases that had not responded to

other drugs. This therapeutic efficacy becomes evident with doses that are usually 3 or 4 times smaller than those of carbutamide and tolbutamide.

In almost all of our case histories the effective quantity of chlorpropamide for prolonged treatment was less than 1 gm./day; in many instances only 0.5 gm., and in several cases 0.25 gm. and 0.125 gm./day were efficacious. This fact bears out the observations made by Root (1958) in the rat, when she showed that chlorpropamide was 20 per cent more active in this animal than carbutamide and 60 per cent more efficacious than tolbutamide.

In all of our cases the blood chlorpropamide concentration was maintained at effective levels and, when the doses were 1 gm. or larger, the figures rose significantly, as already mentioned with reference to the results. In no instance was any definite attack on the hepatic or renal parenchyma or hematopoietic system evidenced although, on two occasions, positive liver flocculation tests and the elevation of alkaline phosphatase observed in patients maintained for a relatively long time on 1 gm. of the compound raised a question as to the harmlessness of the substance to the parenchyma of the liver when administered over a protracted period of time in high dosage (see results among 15 patients in TABLE 9).

In general, the good therapeutic responses in our patients maintained the same relationship with various factors as those reported by Menhert *et al.* (1958) with respect to tolbutamide: the onset of disease in middle age, small previous insulin requirements, short history of diabetes, and recent insulin treatment.

The foregoing bears out the therapeutic inefficacy observed in infantile and juvenile patients and in several of those patients who required insulin doses of more than 30 U., in all of whom the continuation of treatment with both drugs seems indicated only when necessary to attain metabolic stability that cannot be achieved by other means, since not only have we been able to observe a diminution of broad glycemic variations, but also a marked "warding off" of ketosis, which in several cases was readily manifested when insulin alone was used.

In our cases the reduction of insulin dosage among patients who had depended exclusively on this hormone for regulation ranged between 10 and 100 per cent. This bears out similar observations made in humans with respect to tolbutamide by Mirsky and Diengott (1957) and by Fabrykant (1958), which confirm those carried out in the rat with tolbutamide by Houssay *et al.* (1957) and with chlorpropamide by Root (1958).

The increased metabolic stability offered by some oral hypoglycemics has also been pointed out with special reference to carbutamide by Izzo (1957), to tolbutamide by Fulner *et al.* (1956), Bänder (1956), Fabrykant (1958), and to various biguanidines by Krall and White (1958).

It is interesting to note that several patients who were controlled previously with both insulin therapy and later with chlorpropamide claimed that they felt better with the latter substance. This fact was more pronounced in children, even in periods of unsatisfactory compensation.

This feeling of good health made it necessary to be extremely vigilant as to observance of the prescribed diet, for patients might easily commit serious

TABLE 9

Case No.	Hanger test	Thymol turbidity	Kunkel test	Alkaline phosphatase	Cholesterol	Bilirubin	Proteins	Electrophoresis	Hemogram
1	(-)	2.1	5.2	3	262	D = 0.05 I = 0.55 Tot. = 0.6	A = 4.46 G = 2.23 Tot. = 6.69 A/G = 2	A = 3.01 = 45% α_1 = 0.27 = 4.1% α_2 = 0.80 = 12% β_1 = 0.80 = 12.1% β_2 = 0.35 = 5.3% γ = 1.43 = 21.5%	
2	(-)	0.5	2.5	2.64	260	D = 0.1 I = 0.5 Tot. = 0.6	A = 4.62 G = 2.61 Tot. = 7.23 A/G = 1.76	A = 3.75 = 51.9% α_1 = 0.45 = 6.3% α_2 = 0.74 = 10.3% β = 0.93 = 13% γ = 1.31 = 18.2%	
3	(-)	4	6	16.4	202	D = 0 I = 0.4 Tot. = 0.4	A = 4 G = 2.46 Tot. = 6.46 A/G = 1.62	A = 3.30 = 51.2% α_1 = 0.27 = 4.3% α_2 = 0.73 = 11.4% β = 0.74 = 11.5% γ = 1.38 = 21.4%	
4	(-)	3.5	6	2.68	250	D = 0.1 I = 0.6 Tot. = 0.7	A = 4.46 G = 2.54 Tot. = 7 A/G = 1.71	A = 3.74 = 53.4% α_1 = 0.29 = 4.18% α_2 = 0.71 = 10.12% β = 0.66 = 9.43% γ = 1.60 = 22.85%	

TABLE 9 (Continued)

Case No.	Hanger test	Thymol turbidity	Kunkel test	Alkaline phosphatase	Cholesterol	Bilirubin	Proteins	Electrophoresis	Hemogram
5	(-)	2	4.5	6.18	242	D = 0.15 I = 0.65 Tot. = 0.80	A = 4.31 G = 2.77 Tot. = 7.08 A/G = 1.55	A = 3.49 = 49.3% α_1 = 0.22 = 3.2% α_2 = 0.62 = 8.8% β = 1.17 = 16.6% γ = 1.55 = 21.9%	
6	(-)	3.1	5.8	10.1	216	D = 0 I = 0.5 Tot. = 0.5	A = 4.64 G = 2.90 Tot. = 7.54 A/G = 1.60	A = 3.55 = 47.2% α_1 = 0.23 = 3.1% α_2 = 1.17 = 15.6% β = 0.98 = 13% γ = 1.57 = 20.9%	
7	(-)	4.5	10	3.5	230	D = 0.15 I = 0.70 Tot. = 0.85	Tot. = 6.55		R.B.C.: 4,700,000 W.B.C.: 5,900 Hb.: 98%
8	(-)	5	6	1.46	290	D = 0.1 I = 0.58 Tot. = 0.68			R.B.C.: 5,100,000 W.B.C.: 6,900 Hb.: 92% Diff.: N = 59%; E = 7; B = 0; L = 30; M = 4.
9	(+)	4.2	5	2.33	260	D = 0.05 I = 0.61 Tot. = 0.66			R.B.C.: 4,640,000 W.B.C.: 5,900 Hb.: 76% Diff.: N = 62%; E = 4; B = 0; L = 30; M = 2.

10	(++)	2.05	4.5	5.38	230	D = 0.05 I = 0.50 Tot. = 0.55				R.B.C.: 5,080,000 W.B.C.: 6,800 Hb: 96%; V.G. = 0.94 Diff.: N = 72%; E = 3; B = 0; L = 22; M = 3.
11	(++)	3.8	4.2	2.92	315	D = 0.1 I = 0.68 Tot. = 0.78				R.B.C.: 5,050,000 W.B.C.: 10,000 Hb: 14.5 G. Diff.: N = 61%; E = 1; B = 0; L = 33; M = 5.
12	(+++)	17.7	2.3	8.49	1.95	D = 0.15 I = 0.75 Tot. = 0.9				
13	(+++)	5.6	8.5	5.22	206	D = 0.1 I = 0.59 Tot. = 0.69				R.B.C.: 5,580,000 W.B.C.: 8,100 Hb: 16.2 G. = 108% V.G. 0.93 Diff.: N = 62%; E = 0; B = 0; L = 33; M = 5.
14	(-)	1.5	2	3	212	D = 0 I = 0.6 Tot. = 0.6				R.B.C.: 4,900,000 W.B.C.: 7,000 Hb: 12.8 G. = 86% Diff.: N = 69%; E = 2; B = 0; L = 26; M = 3.
15	(+)	3.5	7.2	3.39	262	D = 0.05 I = 0.64 Tot. = 0.69				R.B.C.: 4,700,000 W.B.C.: 6,400 Hb: 12.6 G. = 84% Diff.: N = 67%; E = 1; B = 0; L = 28; M = 4.

dietetic transgressions, especially considering that the experience with these patients of Group 5 reveals the marked increase in tolerance to carbohydrates offered by the compound in milder cases.

According to our experience, chronic infections and cardiovascular conditions that the patients might have do not seem to impair the therapeutic efficacy of chlorpropamide. On the other hand, acute febrile disorders made it necessary in the majority of such cases to resort to the use of insulin.

In accordance with the findings of this study, chlorpropamide does not seem to interfere with the normal functioning of the hypophyseal suprarenal axis or the thyroid function. However, the brief duration of our investigation prevents us from accurately appraising the slight diminution registered in the serum protein-bound iodine values of some patients, which we ordinarily consider the earliest and surest sign of thyroid inhibition (Cardonnet *et al.*, 1957).

While it is not yet possible to claim positive results in cases with special conditions, what we have observed appears to justify the therapeutic testing of chlorpropamide in widely varying diabetic clinical conditions such as insulin allergy and insulin resistance, since in many cases the drug can successfully contribute to improvement of the condition.

SUMMARY AND CONCLUSIONS

(1) This paper describes the studies made of 30 nondiabetics in order to evaluate the hypoglycemic action of chlorpropamide and of 60 diabetics in order to determine its therapeutic efficacy.

(2) It was possible to prove the rapid and pronounced hypoglycemic effect of doses even lower than 500 mg./day and the gradual elimination of the compound from the blood stream, the therapeutically effective blood concentration being estimated at between 3 and 5 mg./100 ml. of blood.

(3) Chlorpropamide proved to be a good therapeutic agent in cases in which other sulfonylureas had also been effective, as well as in some cases that had not responded to either carbutamide or tolbutamide, and in others that required insulin, which it replaced in varying proportions. Its therapeutic scope might be wider than that of other oral hypoglycemics although in general, the same standards of selection already known for other compounds are applicable to chlorpropamide.

(4) The effective therapeutic maintenance dosage was estimated to range between 250 and 750 mg./day and can be administered in single doses or in doses proportioned throughout the day; these quantities are much smaller than the doses of carbutamide and tolbutamide required to obtain equivalent clinical effects.

(5) Untoward side effects, absent with the dosage recommended above, became evident in two thirds of the cases when 1 gm. or more per day was administered. Among these effects the most common were disorders of the upper gastrointestinal tract and facial flushing coincident with the ingestion of alcoholic beverages.

(6) No significant changes were registered in various endocrine functions nor in the functioning of the hepatic gland, the kidney or the hematopoietic

system, although the brief span of observation (3 months) does not make it possible to regard this assertion as definite.

(7) Chlorpropamide can be adapted to the treatment of various clinical conditions such as insulin resistance, insulin allergy, and cases complicated by chronic infections or by other endocrine disorders, since in many of them it can usually, but not always, contribute to improved treatment.

ACKNOWLEDGMENT

The chlorpropamide (Diabinese) used in these investigations was provided by O. H. Solá of Pfizer Argentina S.A.C. e I., to whom the authors express their gratitude.

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BLOOD LEVELS OF CHLORPROPAMIDE IN NORMAL MEN FOLLOWING CHRONIC ADMINISTRATION

Michael Carlozzi, Domenic G. Iezzoni, Lawrence Silver
Chas. Pfizer & Co., Inc., Brooklyn, N. Y.

As part of a large study program on the efficacy and activity of the new oral hypoglycemic agent, chlorpropamide, it was felt that a knowledge of the blood levels at various dosages following chronic oral administration would be of importance. We were already aware that only small amounts are excreted in the urine. Therefore it seemed necessary to determine whether serum levels continued to rise with repeated administration or whether some plateau value would ensue.

Method

Three groups of normal male volunteers were placed on a daily dosage schedule of either 0.25, 0.50, or 1.0 gm. chlorpropamide daily. The group

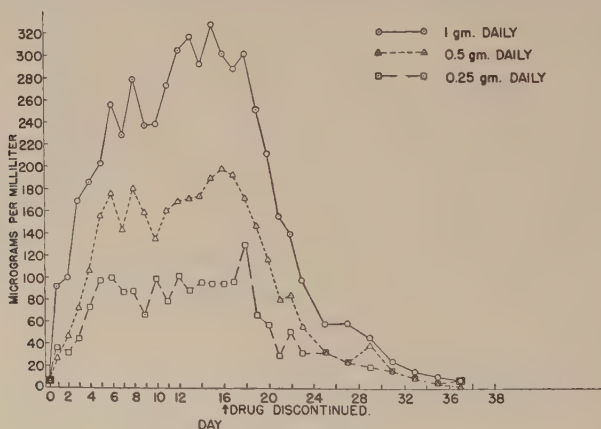


FIGURE 1. Chlorpropamide serum levels.

taking the highest dose was composed of 20 men; the other 2 groups comprised 10 men each. A fasting blood sample was drawn on the morning of the first day and the drug was administered orally immediately afterward. On succeeding days, a 10-ml. fasting sample was drawn in the morning and the drug again administered. This procedure was carried out for 37 days except that, at the end of 15 days, one half of each group on 0.25 and 0.5 gm. was discontinued on the drug while the remaining members of the group received 100 mg. daily. The entire group on 1.0 gm. daily had the drug discontinued at the end of 15 days, and the serum levels were followed through the thirty-seventh day. Usual diets were maintained throughout the study period except for added carbohydrates as needed to relieve sensations of weakness and hunger. Analyses of the serum for chlorpropamide were carried out in our laboratories by the method of Toolan and Wagner.¹

TABLE 1
BLOOD LEVEL OF CHLORPROPAMIDE, 0.25 Gm. DAILY ($\mu\text{g./ml.}$)

Patient	Day 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	25	27	29	31	33	35	37	
21	63	30	41	43	95	87	35	43	35	77	43	90	77	40	63	80	75	75	48	47	18	37	10	0	35	42	0	0	0	0	
22	44	30	40	83	—	75	90	375	—	—	—	—	—	50	48	75	82	250	55	63	25	48	32	—	—	15	30	—	10	10	
23*	55	41	47	78	125	135	120	130	85	112	103	185	158	110	160	138	207	130	125	122	90	110	80	15	77	50	40	32	25	20	
24	45	49	45	78	98	100	77	80	60	75	58	100	75	—	85	93	113	—	—	—	—	—	—	—	—	—	—	—	—	—	
25*	43	42	76	74	113	95	88	130	100	162	92	125	90	123	100	120	110	123	110	125	90	125	50	40	50	50	35	32	25	20	
26*	46	52	52	67	110	115	127	118	70	145	98	107	93	113	105	123	123	105	102	85	70	82	68	—	50	55	45	55	—	25	
27	39	43	41	73	92	110	83	92	70	85	65	50	80	90	97	98	83	82	60	47	30	50	30	85	15	10	18	20	—	10	
28	39	44	68	97	117	103	90	120	40	85	90	100	90	140	123	123	125	107	102	72	38	67	48	10	18	10	—	12	0	0	
29*	40	44	69	62	50	87	75	77	60	80	75	75	60	87	102	113	128	83	55	70	58	70	57	48	42	15	30	20	10	10	
30*	43	40	64	73	85	92	75	100	77	77	75	70	65	98	68	102	85	70	70	48	18	62	47	—	15	40	0	0	0	0	
Mean	46	Mean														no drug															
		42	54	73	98	100	86	88	66	99	78	101	88	95	95	119	131	102	92	90	65	90	60	34	47	42	30	28	15	15	

* Started on 100 mg. daily on sixteenth day; others discontinued.

TABLE 2
BLOOD LEVEL OF CHLORPROPAMIDE, 0.5 GM. DAILY ($\mu\text{g./ml.}$)

BLOOD LEVEL OF CHLOROPROMIDE, U.S. G.M. DAILY (VS. 7.0 mg.)																															
Patient	Day 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	25	27	29	31	33	35	37	
31	15	23	48	63	93	90	85	119	98	110	95	115	110	130	150	123	115	90	90	50	30	25	0	0	0	—	0	0	0	0	
32*	35	62	85	156	115	197	177	235	188	238	235	230	240	263	250	270	293	410	222	162	132	182	155	27	80	67	42	—	—	35	25
33*	30	63	90	122	151	190	173	220	160	185	183	200	200	195	220	250	220	180	180	152	90	125	105	62	50	35	35	—	—	25	20
34*	21	48	80	98	190	115	108	160	165	90	148	100	162	180	185	175	167	150	135	105	88	—	65	55	33	20	12	0	0	0	0
35	20	46	63	123	163	165	142	190	145	130	145	198	200	190	227	220	200	190	165	90	80	75	57	47	10	22	20	0	0	0	0
36	34	57	109	153	230	265	188	245	212	215	233	250	253	260	283	275	233	200	190	150	85	125	77	63	30	32	20	30	0	0	0
37	36	65	110	110	210	190	150	230	200	160	210	223	165	235	193	193	—	220	177	170	115	120	90	—	50	80	27	35	20	10	10
38*	31	49	65	84	123	125	100	127	120	112	110	105	110	—	140	145	123	112	80	90	50	75	57	42	—	50	20	50	20	15	15
39	30	52	78	115	226	250	157	212	240	177	170	192	205	225	187	177	220	150	115	125	90	77	57	10	30	18	—	0	0	0	0
40†	5	0	0	36	60	—	—	63	58	33	75	65	52	80	55	120	83	50	30	30	0	30	—	0	0	0	0	0	0	0	0
															Mean off drug																
																198	192	170	147	117	80	84	56	30	25	38	17	13	4	2	2
Mean	26	47	73	106	156	176	142	180	159	135	160	168	170	173	189	210	201	213	154	127	90	127	96	47	54	43	27	25	20	15	15

* Started on 100 mg. daily on sixteenth day; others discontinued.

† Excluded from mean.

TABLE 3
BLOOD LEVEL OF CHLORPROPAMIDE, 1.0 Gm. DAILY ($\mu\text{g.}/\text{ml}$)

Patient	Day 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	25	27	29	31	33	35	37
1	105	139	214	174	276	302	157	238	198	220	245	277	275	250	310	268	290	275	255	170	62	125	90	30	35	30	18	—	0	0
2	85	106	169	131	248	258	—	268	230	255	257	348	297	355	345	320	407	320	275	208	187	175	130	93	62	72	32	27	15	10
3	92	78	153	232	107	137	173	212	183	175	182	230	223	172	233	232	140	215	237	140	105	85	65	40	15	35	10	0	0	0
4	85	64	142	199	103	155	137	202	190	198	365	288	290	285	295	270	275	295	237	150	145	120	100	50	45	50	30	35	10	10
5	91	91	170	238	115	195	165	263	180	223	205	160	200	—	255	138	200	160	140	125	90	62	40	18	25	15	0	0	0	0
6	100	87	182	208	305	360	338	405	380	315	303	330	295	362	—	315	300	335	298	255	150	182	155	68	70	62	35	27	20	10
7	128	95	201	—	250	243	237	305	215	215	220	250	215	272	—	273	258	277	237	135	77	95	70	—	40	60	30	20	10	10
8	88	95	188	184	265	—	290	243	285	288	270	262	268	302	—	262	290	287	243	187	112	105	75	35	30	25	40	0	10	0
9	93	132	189	238	63	120	127	155	188	205	262	300	325	223	325	280	285	300	195	210	130	110	95	50	50	50	30	20	10	10
10	79	71	134	103	70	295	227	190	274	285	280	348	420	420	398	308	345	375	325	250	200	170	130	38	40	42	15	12	0	0
11	75	87	178	208	275	370	350	425	245	220	325	370	385	328	365	373	363	372	220	307	205	170	105	90	50	48	45	40	20	12
12	82	132	202	224	218	320	325	302	225	260	335	320	360	210	363	313	358	295	312	215	215	125	125	65	47	42	18	0	0	0
13*	58	59	122	92	220	263	185	156	90	100	50	40	20	35	65	50	40	65	62	50	20	48	45	0	40	48	25	0	0	0
14*	0	0	0	0	0	205	230	150	102	45	55	25	15	35	57	20	37	52	47	48	0	37	57	12	12	30	17	10	0	0
15	77	52	90	135	307	370	310	362	225	190	383	350	370	330	202	335	300	257	295	232	170	112	112	60	65	52	15	0	0	0
16	80	138	177	242	255	—	265	—	275	252	275	320	365	325	365	377	348	238	220	187	200	30	65	62	35	12	0	0	0	0
17*	63	111	117	139	157	145	130	110	120	68	—	25	55	70	38	48	48	65	78	48	13	65	43	20	35	60	18	0	0	0
18	98	108	163	196	133	287	245	312	220	243	220	325	323	320	368	310	270	—	285	255	165	148	135	73	77	50	27	—	10	10
19	0	0	0	0	258	318	345	312	328	302	310	365	387	315	385	343	295	298	235	272	220	200	137	77	153	80	35	30	20	20
20	77	159	252	242	—	225	172	258	193	200	180	260	250	200	262	273	205	—	193	160	98	105	48	18	—	0	0	0	0	0
Mean	92	100	169	186	202	255	228	278	237	238	272	304	315	292	327	301	287	301	250	211	155	139	98	57	59	45	24	14	8	5

* Excluded from mean; all drugs stopped on sixteenth day.

Results and Discussion

The average daily serum levels of chlorpropamide for the three dosages administered are given in FIGURE 1. TABLES 1, 2, and 3 show that the individual serum levels within each group were spread over a considerable range

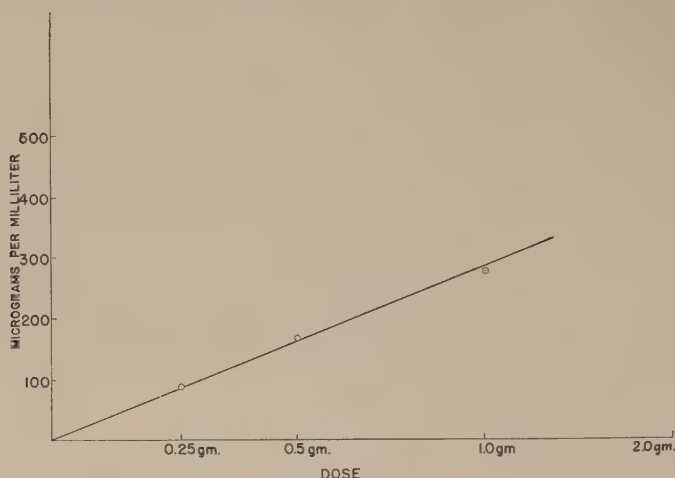


FIGURE 2. Log-dose chlorpropamide serum level curve.

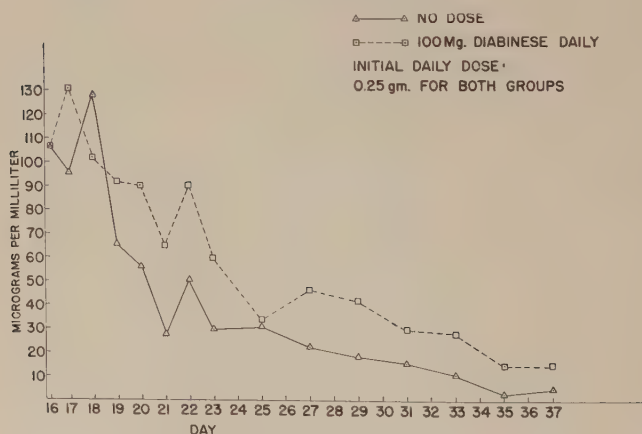


FIGURE 3. Effect of chlorpropamide—100 mg. daily—on maintenance of serum level.

of values. However, each subject, on a day-to-day basis, had reasonably consistent values. It is apparent that at 0.25 and at 0.50 gm. daily the serum levels reach a plateau in 3 to 5 days around which there is some daily variation. At 1.0 gm. daily it appears that the plateau is reached somewhat later; there appears to be a further cumulative effect after 5 to 6 days. Averages of the plateau levels for each dose yielded a straight line log-dose curve as shown in FIGURE 2. The rate of excretion of chlorpropamide is con-

siderably slower than that for tolbutamide and, presumably, accounts for the more prolonged effect that has been reported. FIGURES 3 and 4 demonstrate that chlorpropamide given at a dose of 100 mg. daily is insufficient to maintain the serum levels of chlorpropamide reached with prior daily dosage at levels of 0.25 gm. and 0.5 gm. Furthermore, the rate of decline of serum chlorpropamide levels is substantially the same in those individuals who were maintained on chlorpropamide at 100 mg. daily after discontinuing the prior larger daily doses as in those where the medication was discontinued

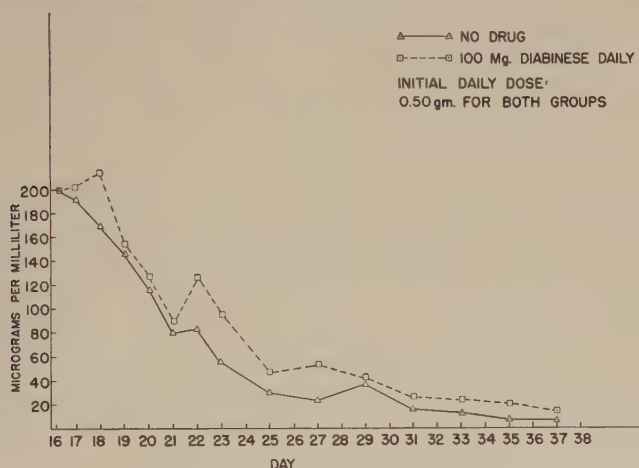


FIGURE 4

after 15 days. During the course of the study, there were no subjective or objective symptoms observed. However, many of the subjects did experience hunger and supplemented their diet with chocolate and hard candies.

Summary

Various doses of chlorpropamide were given orally to normal volunteers. Serum levels were followed for thirty-seven days. No untoward effects were observed.

Reference

1. TOOLAN, T. & R. L. WAGNER, JR. 1959. The physical properties of chlorpropamide and its determination in human serum. *Ann. N. Y. Acad. Sci.* **74**(3): 449.

SULFONYLUREA DRUGS IN THE TREATMENT OF DIABETES MELLITUS

P. B. Landabure, C. Delbue, C. Alvariñas, N. Serantes,
R. Camerini Dávalos, Gregorio Waisman, A. Cabarro, E. Dussel,
F. Cicchitti, A. Loredo

National Institute of Nutrition, Buenos Aires, Argentina

INTRODUCTION

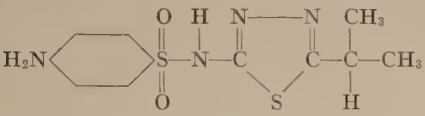
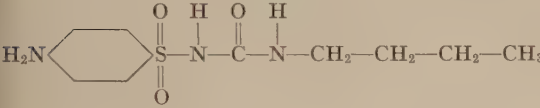
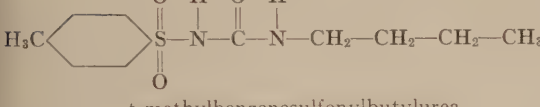
The National Institute of Nutrition of Argentina first began clinical investigation of the sulfonylurea drugs with carbutamide in March 1956. The results were reported at a meeting of the Argentine Association of Nutrition and Dietetics in October 1956 and were published in 1957.¹ We expanded our work to include tolbutamide, and a report of the results obtained with this drug was also published.² Some of us have reported separately on our experiences with the sulfonylurea drugs.³⁻¹¹ Two conferences were held on sulfonylurea drugs by the Argentine Medical Association (in 1957 and 1958) and two special sessions were held by the Argentine Association of Nutrition and Dietetics.

Theoretically, the ideal sulfonylurea drug would provide the following: (1) minimal gastrointestinal intolerances, with no acute or chronic side reactions or toxicity and an ample margin of safety; that is, a wide difference between the therapeutic dose and the toxic dose; (2) a favorable action in the majority of diabetics, regardless of age, physical condition, nutritional status, type of diabetes, stage of the disease, complications, or intercurrent disease; and (3) a mechanism of action that is closest to the normal physiological mechanism that maintains sugar metabolism.

An analysis of all available preparations reported in the literature was published by one of us in June 1958⁶ and is summarized in TABLE 1. It is evident from the ample literature of the past three years that we are far from achieving the ideal therapeutic agent. For this reason, the appearance of any hypoglycemic drug makes it mandatory that we determine how close it comes to the therapeutic ideal and establish a comparison with insulin and drugs previously evaluated in regard to efficacy, dosage, intolerance, and possible future effect on the therapy of diabetes.

With this point of view in mind, the National Institute of Nutrition, the Chair of Pathology, and the Nutrition Clinic, in association with other Argentine centers for the study of diabetes situated in La Plata, Mendoza, and Córdoba, carried out a study of the clinical effects of chlorpropamide. The method involved rigid conformance to the same techniques at different places, which invests this project with both a unity and a diversity that affect the significance of the results.

TABLE 1
 CONSENSUS ON ACTION OF AND INDICATIONS FOR BENZENESULFONAMIDES*

Benzenesulfonamides	Probable locus or mode of action
 <p><i>p</i>-aminobenzenesulfamidoisopropylthiodiazole</p>	(1) Pancreas
 <p><i>p</i>-aminobenzenesulfonylbutylurea</p>	(2) Inhibition of insulin degradation (3) Liver enzyme systems
 <p><i>p</i>-methylbenzenesulfonylbutylurea</p>	(4) Peripheral effect on carbohydrate metabolism
Criterion of case selection	Factors indicating use of benzenesulfonamides
(1) Age (2) Duration of diabetes (3) Duration of insulin treatment (4) Insulin dosage (5) Constitutional type (6) Fasting blood sugar level before treatment (7) Ketosis tendency	(1) More than 50 years old (2) Less than 10 years (3) Less than 5 years (4) Less than 40 U. (5) Pyknic, with obesity and essential hypertension tendencies (6) Not more than 300 mg./cc. (7) None

* To June 1958.

MATERIAL AND METHODS EMPLOYED

Characteristics of the Patients Studied

The drug was used on 200 patients, but data have been tabulated for only 130; these patients have been treated for a sufficient length of time to permit an evaluation to be made (FIGURE 1).

Conditions Under Which the Study Was Conducted

Form of control. Of the total of 130 patients whose data have been tabulated, 30 (23 per cent) were studied while they were hospitalized, and 100 (77 per cent) were observed in the outpatient department. The latter were

selected from patients who assured us that they would follow instructions carefully. To evaluate fully the effect of the drug we administered it to patients having various types of diabetes. After observations of responses were made, we continued our investigation of those individuals who had some degree of satisfactory response to the drug.

The diet followed. A nutritional study was conducted in order to acquire a knowledge of the diet of all patients. A dietary interview was held with each patient during which a detailed history of his dietary habits was recorded.

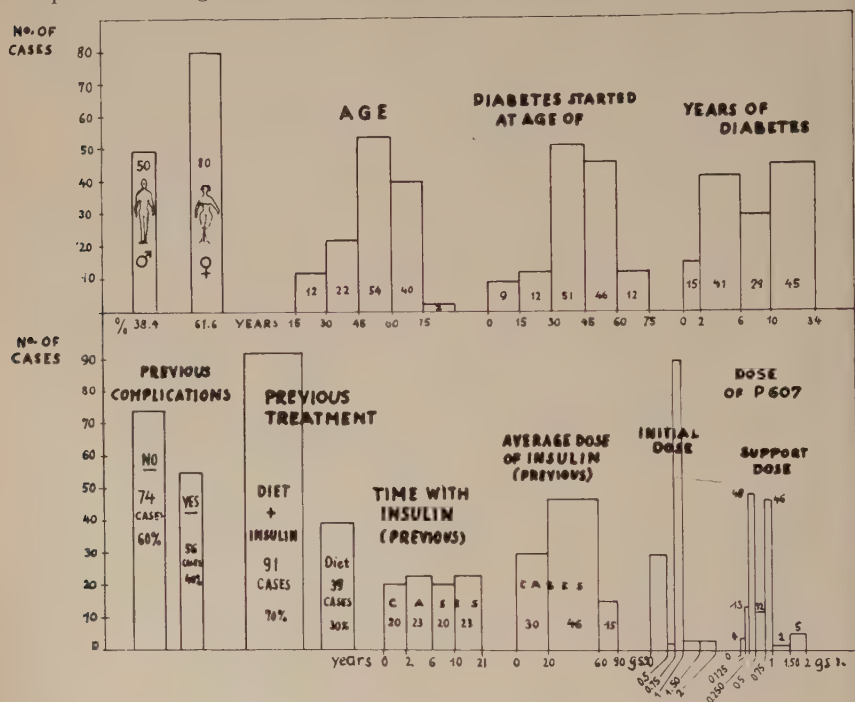


FIGURE 1. Characteristics of 130 cases under observation.

Dietary regimens, with the characteristics of these normal regimens introduced, were designed to provide each patient with the caloric value appropriate for his or her nutritional status, and to include approximately 200 gm. of carbohydrates; this amount was reduced in certain cases and increased in others to as much as 300 gm. It is understood, of course, that the other values—those of the minerals and vitamins—were the same as in the normal regimen in every case.

Examinations and tests used to evaluate the response to therapy. The observation of each patient in the study was carried out under strict clinical, medical, and laboratory control. Clinical determinations included body weight, diuresis, and any subjective or objective symptoms that appeared. Laboratory determinations included blood sugar before breakfast, blood sugar profile during the day, total daily urine sugar and urine sugar profile, exist-

ence of acetonemia, complete urinalysis, protidemia, lipemia, urea in the blood, liver function tests, hemogram, renal adequacy test and, in a sizable number of cases, liver biopsy puncture.¹² In the hospitalized patients, a study of the carbohydrate metabolism was performed with the standard curves (Allbright's technique), intravenous glucose, intravenous insulin glucose, and adrenalin insulin. In six cases the action of chlorpropamide in intravenous administration was studied.

Dosage Schedule for Chlorpropamide

The drug was administered in most of the cases in 2 doses (1 after breakfast and 1 after lunch). In some patients the drug was given after dinner. The highest total dosage administered thus far is 71 gm.; the longest duration of therapy, 130 days. All patients are continuing on their respective dosage schedules.

Techniques and Standards Used

Addis counts were made by the usual technique. Blood tests were made as follows: urea, modified enzymatic technique of Wrenn; glucose, the

TABLE 2
DEFINITIONS OF STANDARDS OF CONTROL AND EVALUATION FOR CHLORPROPAMIDE

Control	Blood sugar, mg. % (Somogyi-Nelson)	Total urine sugar, gm.	Acetone
Excellent.....	80-110	none	none
Good.....	111-130	To 5 per cent of the food glucides	none
Fair.....	131-170	To 10-20 per cent of the food glucides	none
No control.....	Higher levels	Higher levels	—
Efficient.....	Produces excellent or good control		
Partially efficient..	Produces poor control. Reduces insulin use no less than 50 per cent. Has stabilizing action on glucose homeostasis.		
Inefficient.....	Apparently no action on diabetes.		

Somogyi-Nelson technique; alkaline phosphatase, the Bodansky method; blood proteins, the techniques of Calvary, Cohn, Wolfson, and Ichiva; cholesterol, the method of Bloor, Sheftel, and Hill; Maclagan, the original technique with adaptation of electrophotometric reading; Hanger, the original technique, including maintaining in the dark for 48 hours; Kunkel, the original technique; blood bilirubin, the technique of Mallory and Evelyn; erythro sedimentation, the technique of Westergren; and the cytological test, the usual technique.

Designations for standards of effectiveness of chlorpropamide in the control of diabetes in relation to blood sugar and glycosuria are shown in TABLE 2. Diabetic groupings according to response to chlorpropamide are shown in TABLE 3.

TABLE 3
CLASSIFICATION OF DIABETICS

Group	Definition
1	Controllable with proper diet and insulin of as much as 20 U. Insulin withdrawal does not produce ketosis.
2	Controllable with proper diet and insulin dosage of as much as 59 U. Insulin total withdrawal, even if less than 20 U., produces ketosis. History of ketosis exists.
3	Controllable with proper diet and more than 60 U. of insulin. With or without past history of coma.

RESULTS

General

The effects of chlorpropamide on blood sugar levels in normal individuals are shown in FIGURE 2; FIGURE 3 shows the effect on blood sugar levels of diabetics; and FIGURE 4 the effect on the control of diabetes as indicated by blood sugar levels and glycosuria.

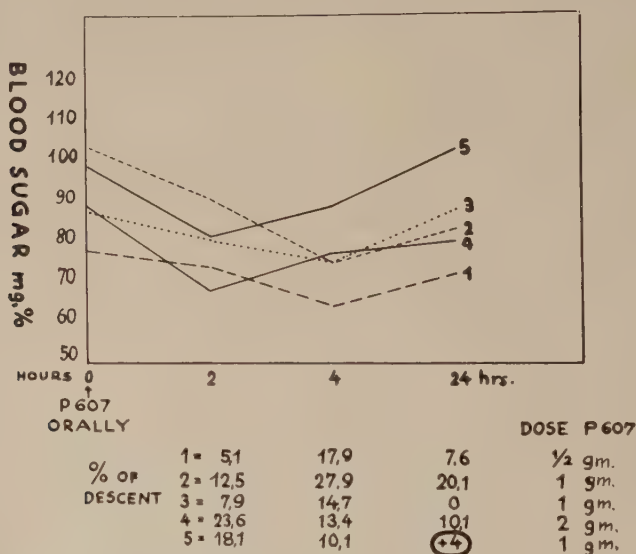


FIGURE 2. Glycemia response with chlorpropamide in five normal subjects.

Special

The complementary action of chlorpropamide on the control of cases of diabetes treated with insulin is illustrated in FIGURES 5 to 8. FIGURE 6 shows the control exercised by tolbutamide and chlorpropamide in diabetics undergoing surgery. FIGURE 7 shows the action of chlorpropamide in two

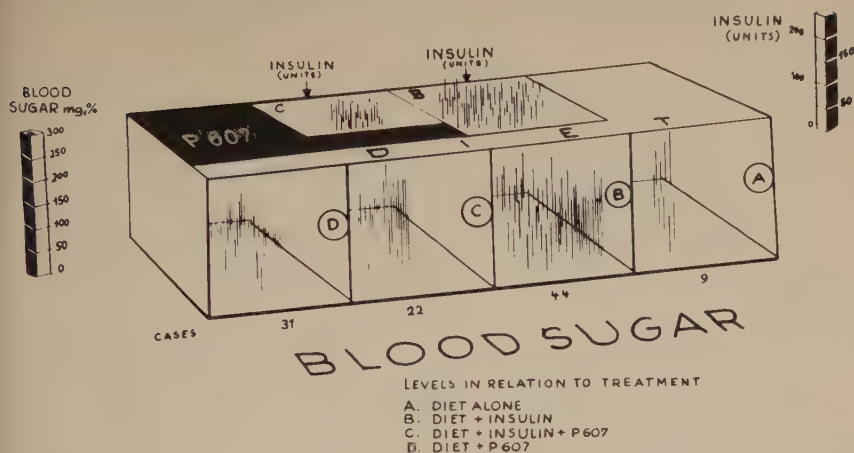


FIGURE 3. Blood sugar levels in relation to treatment.

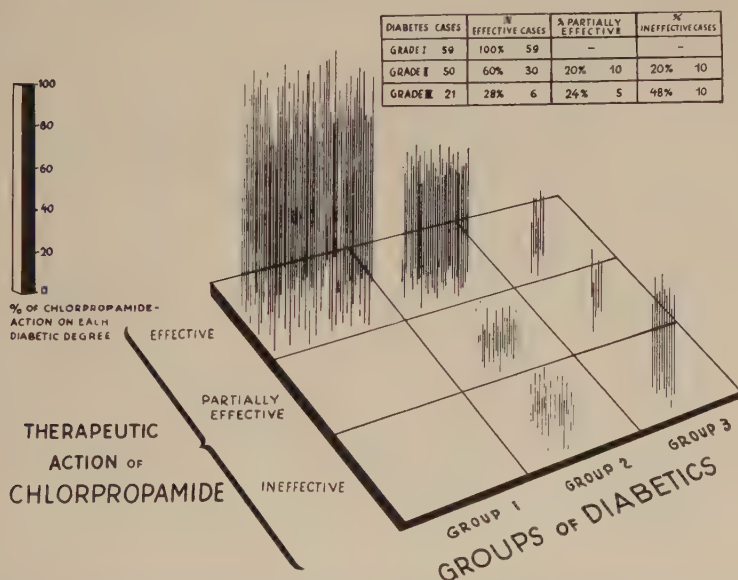


FIGURE 4. Results in 130 diabetic patients treated with adequate diet and chlorpropamide for 4 months.

cases of pancreatic disease—one chronic pancreatitis with steatorrhea and diabetes; the other with acute pancreatitis, operated for obstructive hepatitis (cholecystojejunostomy) three years before the beginning of diabetes. The action of chlorpropamide in one case of hyperthyroidism in diabetes is shown in FIGURE 8. Separate observations were made of the effect on blood sugar levels of methylthiouracil, methylmercaptoimidazole, carbutamide, chlorpropamide, and chlorpropamide with methylthiouracil. In one case of

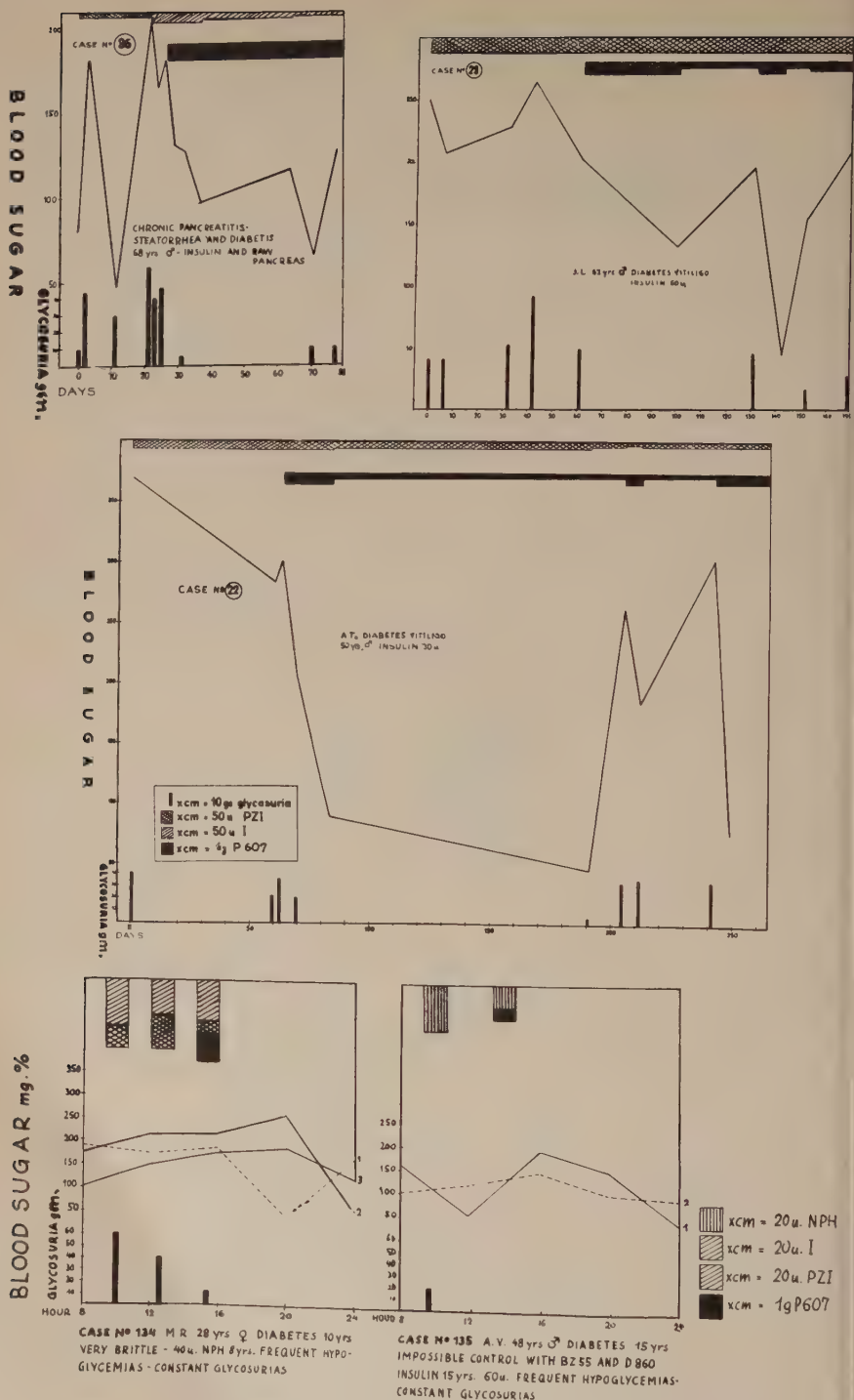


FIGURE 5. Complementary action of chlorpropamide in insulin-treated diabetics.

diabetes and vitiligo, good control was possible with chlorpropamide (FIGURE 9). The effect on blood sugar level of chlorpropamide administered intravenously is shown in FIGURE 10.

No beneficial effect was observed from chlorpropamide administered to two cases of moderate to intense acidosis. Three cases of mild ketosis were correctable with the drug.

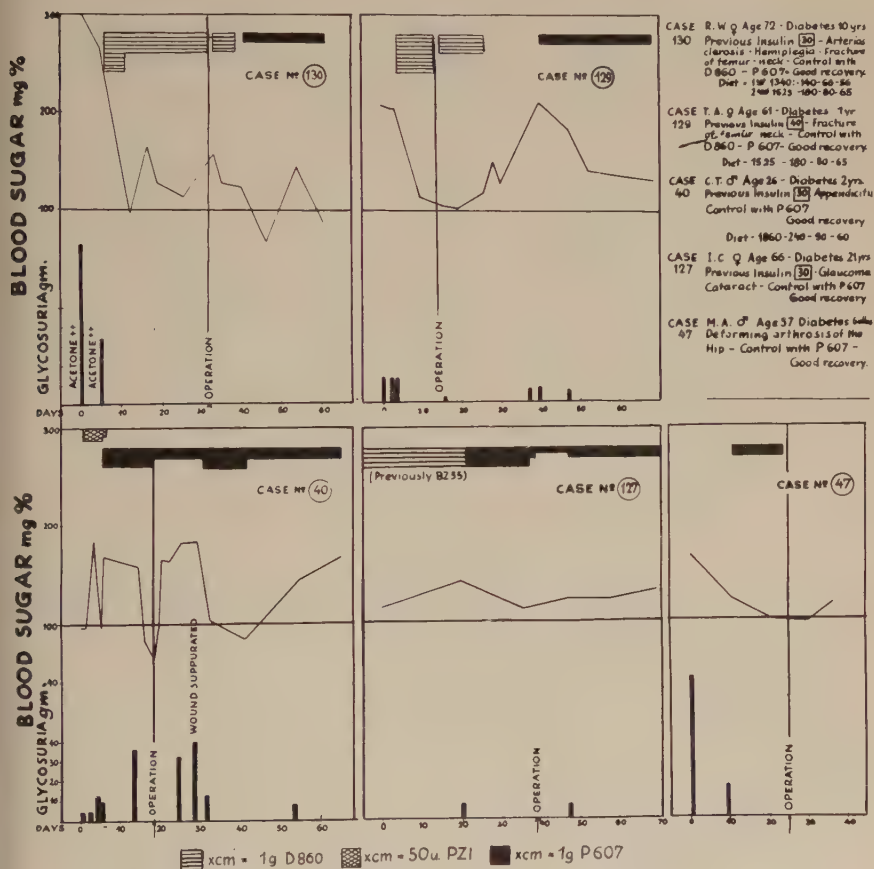


FIGURE 6. Surgical cases treated with tolbutamide and chlorpropamide.

During treatment with chlorpropamide, relatively high blood sugar levels were observed without glycosuria or with only slight glycosuria. This apparent elevation of the renal threshold is accompanied by a decrease in diuresis.

In two cases we have observed a relationship between dosage and degree of control and between body weight and degree of control, with maintenance of a constant dosage of chlorpropamide (FIGURE 11).

In another case a relationship was observed between the type and dosage

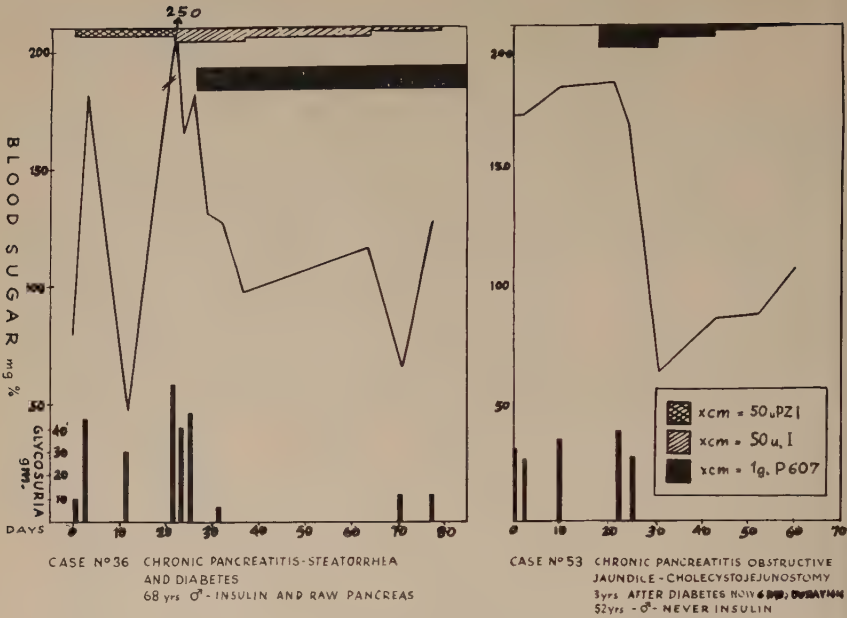


FIGURE 7. Action of chlorpropamide on two cases of pancreatitis.

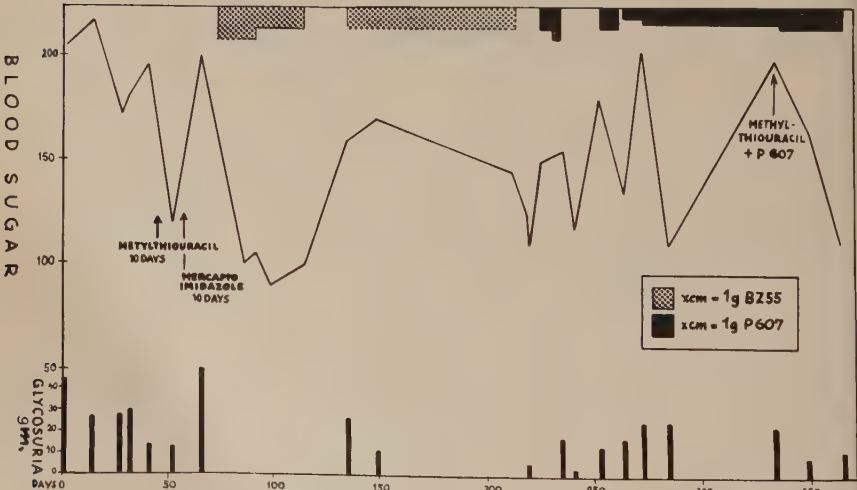
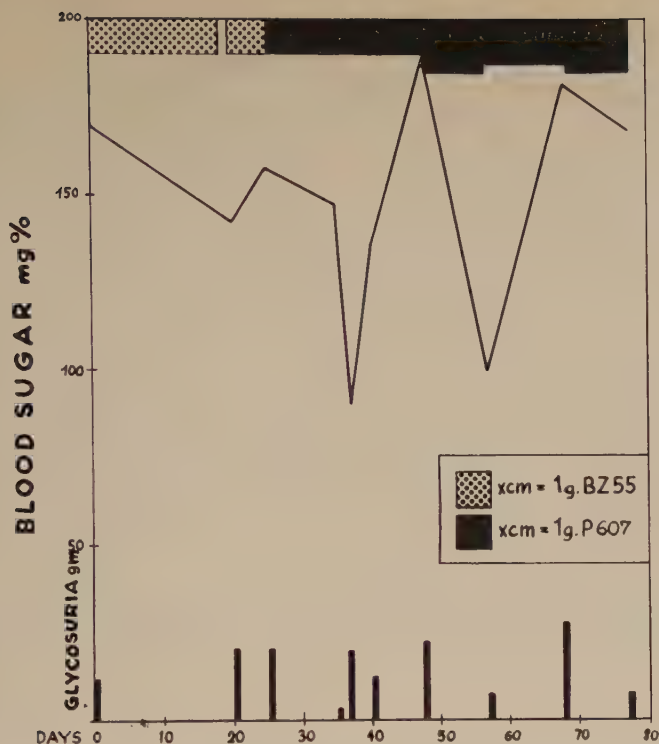


FIGURE 8. Reaction of J. M., 43-year-old male with hyperthyroidism and diabetes, to treatment with methylthiouracil, mercaptoimidazole, carbutamide, chlorpropamide, and chlorpropamide with methylthiouracil. Weight, 70 kg.; ideal weight, 70 kg. A 2000-cal. diet consisted of 240 gm. carbohydrate, 90 gm. protein, and 75 gm. fat.



CASE No 42 A.P. 16yr. diabetes vitiligo age 60 yr
 ♂ Δ - BZ55 2gms P607 1g.

FIGURE 9. Action in relation to dosage.

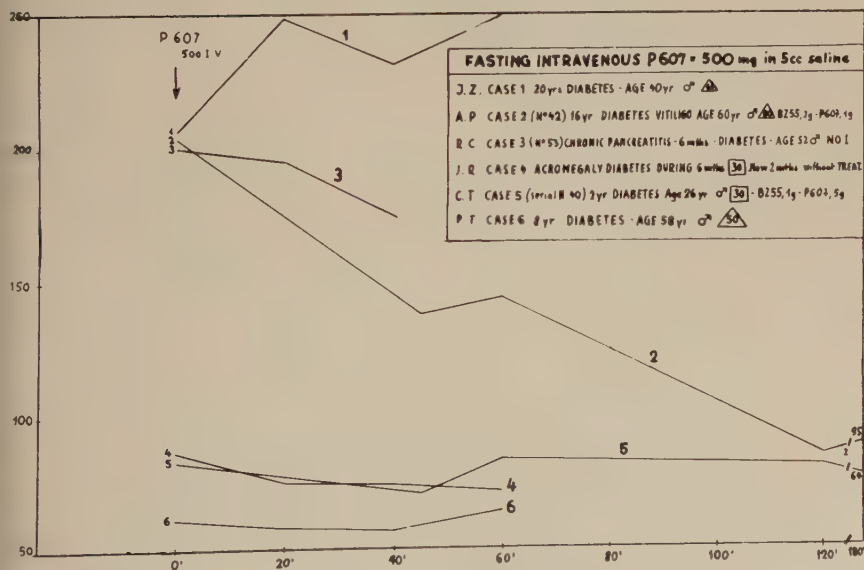


FIGURE 10. Effect on blood sugar level of intravenous administration of chlorpropamide.

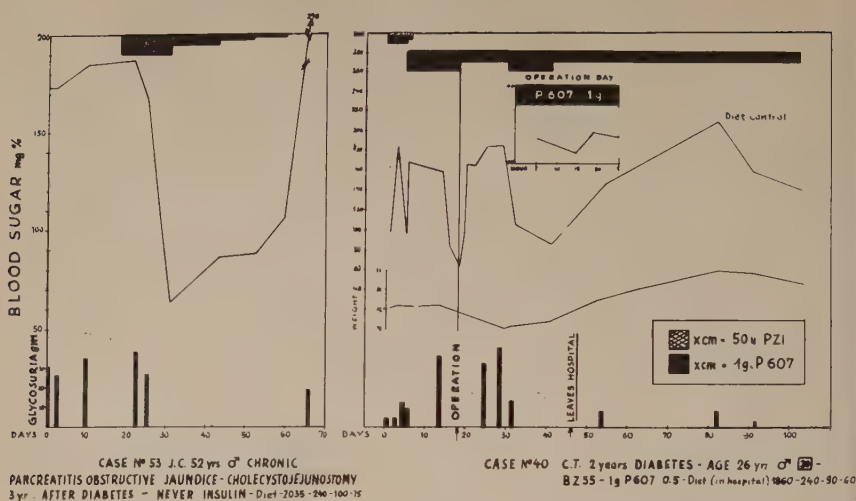


FIGURE 11. Action of chlorpropamide in relation to body weight and dosage.

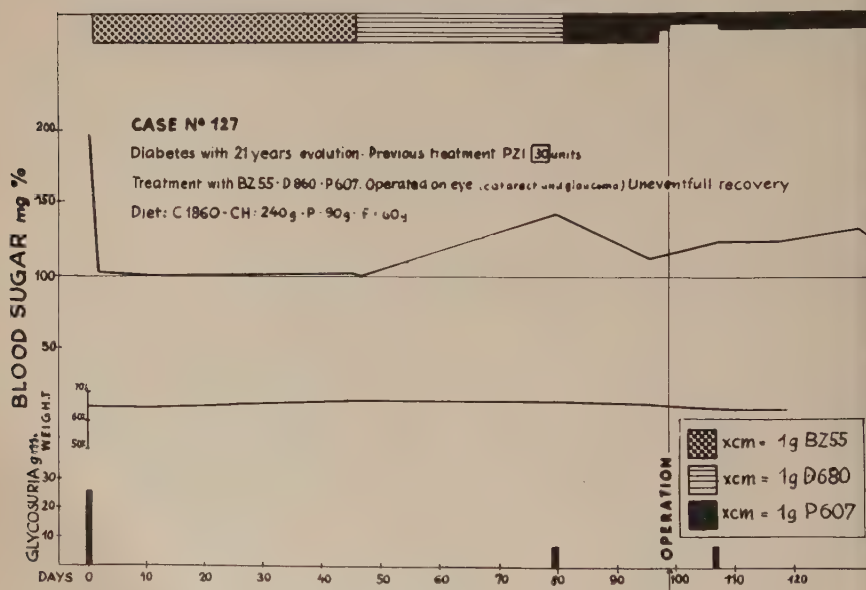


FIGURE 12. Action of drugs in patient whose body weight was kept constant.

of the drug used and the degree of control in a patient in whom the body weight was maintained at a constant level (FIGURE 12).

Side Effects in the Administration of Chlorpropamide

There was 1 gastrointestinal case (constituting less than 1 per cent), and no renal or hepatic cases. Hematic effects were limited to 2 cases of leuko-

penia (1.6 per cent) and cutaneous effects to 15 cases (11.5 per cent), 13 of flushing (10 per cent) and 2 of pruritus with exanthema (1.6 per cent). The flushing observed appeared similar to that produced by the ingestion of nicotinic acid. In two cases where the flushing was intense, it was related to the ingestion of alcohol; when it was reduced, the effect disappeared. The flushing was interpreted as due to a vasodilating effect and not to an allergic reaction. There was 1 case (0.8 per cent) of intense headache and 1 case (0.8 per cent) of cyanosis that disappeared spontaneously and did not reappear when dosage was decreased from 0.5 gm. to 0.25 gm. of chlorpropamide. There were 4 cases (3 per cent) of hypoglycemia; these patients presented symptoms of nocturnal hypoglycemia, which disappeared on reducing the dosage of chlorpropamide. Occasionally other patients showed symptoms of a mild hypoglycemia, but these cases were transitory and could not be classified definitely as actual hypoglycemic episodes. In one case (patient 26) epigastric and precordial discomfort was experienced 4 hours after ingestion of 0.5 gm. of chlorpropamide; this coincided with a clearly low blood sugar.

Side effects were related to dosage. They appeared when the dosage was 1 gm. daily or higher, and rarely with dosage of 0.5 gm. daily (case 89, cyanosis). We have the impression that the margin between the therapeutic dosage and the dosage that produces side effects is small; in other words, the margin of safety is narrow.

DISCUSSION AND CONCLUSIONS

Classification of Diabetes

From our experience with sulfonylurea drugs and, particularly, with chlorpropamide in the treatment of diabetes, it is possible to subdivide diabetics into three groups. Our classification is based on symptoms that are obvious and reliable. After studying all symptoms that can be used for classification, it was decided that the most constant, in relation to the results obtained, are ketosis, diabetic coma, and previous insulin dosage required before establishing therapy with the sulfonylurea drugs. On the basis of the patients' responses with respect to these three factors, we have defined the three groups of diabetics. This manner of grouping has been useful in predicting the results of treatment with the sulfonylurea drugs; furthermore, it facilitates the comparison of results achieved with the various sulfonylurea drugs. The criteria for including a diabetic in a specific group are shown in TABLE 3.

TABLE 4 shows the control achieved with other sulfonylurea drugs as observed in earlier studies carried out with the same methods used with chlorpropamide.

Administration of the Drug

The minimum effective maintenance dosage of chlorpropamide was 0.125 gm. (in 4 cases) to 0.5 gm. per day; the latter was the most commonly used dosage. One-half gram daily was effective in 38 cases. To be effective, it

was necessary to increase dosage to 1 gm. daily in another 33 cases. When it was necessary to increase the maintenance dosage further, 1.5 gm. was effective in 2 cases out of 3, and partially effective in 1 other. In 7 cases in which the drug was ineffective, the dose was increased to 2 gm., with some improvement. With chlorpropamide, the glucose-regulating effect is some-

TABLE 4
RESULTS WITH CARBUTAMIDE OR TOLBUTAMIDE IN 130 DIABETICS

Diabetic group	Carbutamide					
	Effective		Partially effective		Ineffective	
	Percent-ages	No. of cases	Percent-ages	No. of cases	Percent-ages	No. of cases
1	73.80	31	9.52	4	16.66	7
2	68.05	49	15.27	11	16.66	12
3	18.75	3		0	81.25	13

Tolbutamide						
1	61.42	43	17.14	12	21.42	15
2	48.07	25	34.61	18	17.30	9
3	37.50	3	25.00	2	37.50	3

times delayed for as long as 1 week or 10 days, during which time the patient should be observed carefully to determine if a change in dosage is necessary.

Mode of Action

The investigation as conducted does not give any clue to the manner in which chlorpropamide acts. Its action appears to be similar to that of other sulfonylurea drugs, although effective dosage with chlorpropamide is lower. Obviously, one can not be too categorical in comparing dosages of the three sulfonylureas, since little attention was given to reducing the dosages of carbutamide and tolbutamide to minimal levels during previous investigations.^{1, 2} However, under the conditions of our investigation, chlorpropamide appeared to be effective in smaller doses than those required with the other drugs. Experience gained with the sulfonylurea drugs suggests that overdoses of insulin in the treatment of diabetes have been common.

Our investigations do not determine definitely all possible effects of long-term therapy.

We have used chlorpropamide for five months, a rather short time; therefore we can reach only very preliminary conclusions.

Tolerance of Chlorpropamide

The tolerance of effective dosage has been good, as the percentages of intolerance and of side effects indicate. Gastrointestinal intolerance increases with dosages greater than 1 gm. daily.

Usefulness of Chlorpropamide

When this sulfonylurea drug is indicated, it is very effective in reducing high levels of blood sugar to normal, and also is useful in helping to establish normal levels when given in conjunction with insulin.

Treatment with Chlorpropamide and the Importance of Dietary Measures

Regardless of the group in which a diabetic is classified, a basic factor in the treatment is a balanced diet that normalizes body weight. It has been

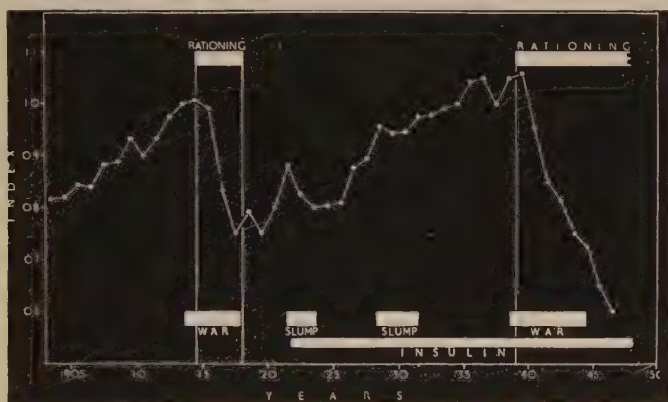


FIGURE 13. Comparative mortality indexes for diabetic patients in England and Wales.

observed that, with chlorpropamide as with other sulfonylurea drugs, stability is endangered when the patient's weight increases. Dramatic evidence of the highly significant importance of this fact is irrefutably presented by the mortality curve for diabetic patients observed since 1900 in England and Wales¹³ (FIGURE 13). Few people seem to have realized the significance of the fact that during World Wars I and II, when rationing was in effect, the mortality rate for diabetics dropped to a marked degree. No more graphic evidence of the importance of adhering strictly to dietary regimens exists.

SUMMARY

Chlorpropamide was studied at the National Institute of Nutrition with the collaboration of diabetes centers of La Plata, Córdoba, and Mendoza. The medication was administered to a total of 200 diabetic patients, all more than 15 years of age. Thus far, however, detailed tabulations have been completed on only 130 of these cases. Patients were subdivided into 3 classifications.

Group 1, those controlled with insulin, dosage of as much as 20 U., but in whom withdrawal of insulin did not produce acidosis.

Group 2, including (a) those controlled with less than 20 U. of insulin, but in whom acidosis appeared on withdrawal; (b) those requiring between 21 and 59 U. of insulin; and (c) those presenting a history of acidosis.

Group 3, those requiring more than 60 U. of insulin or having a history of diabetic coma. Using this classification of diabetic disease, the results shown in TABLE 5 were observed with chlorpropamide in 130 patients of this series.

TABLE 5
RESULTS WITH CHLORPROPAMIDE IN 130 DIABETIC PATIENTS

Diabetic group	Total cases	Effective		Partially effective		Ineffective	
		Percent-ages	No. of cases	Percent-ages	No. of cases	Percent-ages	No. of cases
1	59	100	59	—	—	—	—
2	50	60	30	20	10	20	10
3	21	28	6	24	5	48	10

Hypoglycemic action has been demonstrated with chlorpropamide in normal individuals. As for comparing its efficacy with carbutamide and tolbutamide: equal hypoglycemic action was obtained with smaller doses of chlorpropamide. Otherwise, the same factors appear to govern the effectiveness of these drugs. Daily dosage ranged from 0.1 to 2.0 gm. most frequently; an initial dose of 1.0 gm. (67 per cent of the cases), with 0.5 to 1.0 gm. (70 per cent) for maintenance, proved effective. Side effects included flushing of the skin (10 per cent of the patients), epigastric distress (10 per cent), vomiting (2 per cent), fever (2 per cent), and asthenia (2 per cent). These effects were more common when doses of more than 1.0 gm. were used. Tests of hepatic and renal function revealed no disturbance, even in those patients receiving the drug for as long as 130 days. The action of chlorpropamide has been studied in relation to a diet that contains less carbohydrate than a normal diet.

ACKNOWLEDGMENTS

We express our appreciation of the collaboration of Boris Rothman, Vicente Osvaldo Perrone, Enrique Benito De Marco, Mario Calvagno, Lidia P. de Esquef, and R. S. de Hurst of the National Institute of Nutrition. We thank Oscar H. Solá of the Pfizer Argentina Laboratories for supplying the chlorpropamide used and for his cooperation and assistance.

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PRELIMINARY CLINICAL AND EXPERIMENTAL STUDIES WITH CHLORPROPAMIDE IN DIABETES MELLITUS

Ewen Downie, Joseph Bornstein, Harald Breidahl

The Diabetic and Metabolic Unit, Alfred Hospital, University of Melbourne, Melbourne, Australia

The purpose of this communication is to describe our experiences in the clinical management of sixty diabetic patients who have been treated with chlorpropamide for periods varying from a few weeks to six months. In addition, the results of some comparative experiments on the action of tolbutamide and chlorpropamide *in vitro* are given. These suggest that the mode of action of the two drugs upon hepatic enzyme systems is similar, but there seems to be a quantitative difference between them.

Clinical observations have been made on sixty adult diabetic patients: twenty-one males and thirty-nine females. In view of earlier experiences with tolbutamide and carbutamide, no attempt has been made to treat any juvenile patient; with few exceptions, the persons included in this series have been middle-aged or elderly. Within these limits a random selection has been made from patients attending the routine practice of the diabetic service of this hospital.

In contrast with earlier clinical trials conducted in the Diabetic and Metabolic Unit of the Alfred Hospital,^{1, 2} many of the patients in this series were not admitted to hospital for continuous observation. It was felt that experience gained during the past three years was sufficient to enable us to assess the efficacy of chlorpropamide without the need for hospitalization. In consequence, a number of patients were instructed to present the results of urine tests taken at regular times each day, and spot blood sugar estimations were made at frequent intervals. Subsequent interrogation enabled us to detect the incidence of side effects and the degree of control achieved. A significant number of patients were closely observed, and laboratory investigations were undertaken for evidence of hepatic, renal, and hematological abnormalities. Patients transferred from maintenance doses of insulin were instructed to report daily either in person or by telephone and to present the results of urinalyses performed four times each day.

Almost without exception the loading dosage prescribed was 1.0 gm. per day and the maintenance dose 0.5 gm. per day, given in divided doses of 0.25 gm. morning and evening. In any case that failed to respond, the dosage was raised gradually to 0.75 gm. per day and, in a few instances, to 1.5 gm. per day. Dosage for 1 case was raised to 3 gm. per day for a short time. FIGURE 1 shows the duration of treatment of the patients considered in this series and indicates the number responding to treatment and the number failing to do so. The relatively large number not responding within the first 4 weeks of treatment can be related to several such factors as inappropriate selection of patients, and attempts to transfer cases from insulin therapy. TABLE 1 shows the distribution of the patients by age and sex

and TABLE 2 shows the over-all results obtained in the entire series. It is convenient to present the results obtained for 4 categories of patients: (1) previously untreated, (2) previously treated by dietary restriction, (3) previously treated by diet and tolbutamide, and (4) previously treated by diet and insulin.

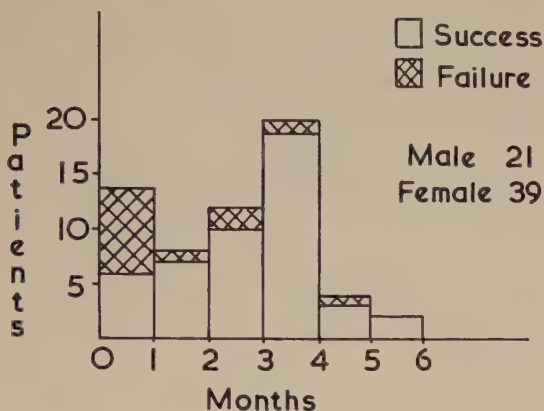


FIGURE 1

TABLE 1
DISTRIBUTION OF PATIENTS BY AGE AND SEX

Age	Male	Female
20-30		1
30-40		1
40-50	2	1
50-60	4	5
60-70	9	15
70-80	4	13
80—	2	4
	21	39

(1) Fourteen patients previously untreated were placed upon an appropriate restriction of diet with chlorpropamide in an initial dosage of 1.0 gm. and a maintenance of 0.5 gm. per day. A number of these were assessed as likely to require insulin for control. Twelve patients responded satisfactorily, while 2 failed to do so.

(2) Twenty patients who had shown inadequate or intermittently satisfactory response to dietary restriction for periods from a few months to several years were treated along similar lines. Seventeen are considered to have shown a satisfactory response to chlorpropamide therapy, while 3 failed to do so.

(3) Four patients who had been treated for periods from 1 to 4 months

with dietary restriction and with tolbutamide in conventional dosages of 1.5 gm. per day, and all of whom had failed to show satisfactory response to this drug, were transferred to chlorpropamide in dosages of 0.25 gm. twice daily. Three showed a satisfactory control, while 1 failed to respond.

(4) Twenty-two patients whose condition had been regarded as controlled more or less satisfactorily by dietary restriction together with varying dosages of insulin were transferred to chlorpropamide therapy. Successful control was established in 15, while 7 failed to respond.

Chlorpropamide was used intermittently for periods of a few days to 1 to 2 weeks in treating still another group of 12 patients who, for various reasons, had become unstable while on dietary restriction alone. It was felt that a short trial of a sulfonyl drug might restore control and that the drug might

TABLE 2
RESULTS OBTAINED IN ENTIRE SERIES

Previous treatment		Success	Failure
Nil.....	14	12	2
Diet alone.....	20	17	3
Diet plus tolbutamide.....	4	3	1
Diet plus insulin.....	22	15	7
	60	47	13

then be discontinued. In every instance the results obtained were considered entirely satisfactory as, after a short course of dosages of 0.25 to 0.5 gm. per day, it was possible to discontinue its use, the patients being restored to their previous state of equilibrium.

TABLE 3 gives some details of the 13 patients who failed to respond. Two patients previously untreated, one a male of 54 and the other a female of 64, both with a history of diabetes of short duration who on general principles were considered likely to respond favorably, showed no evidence of control on dosages of 1.5 gm. and 1.0 gm., respectively, although these dosages were continued for more than a fortnight. In neither case was there any evidence of an appreciable fall in the level of blood sugar.

In three instances, patients whose dietary control had been regarded as unsatisfactory for several months were tried with chlorpropamide therapy; in no instance did it induce improvement. These patients were a female of 73, a male of 65, and a female of 72 who were treated for 6 to 8 weeks with no appreciable improvement. Each of these patients was subsequently satisfactorily controlled with insulin.

Seven of the 22 patients previously taking insulin failed to show satisfactory control when transferred to chlorpropamide. In 5 of these the duration of diabetes ranged from 6 to 10 years and their daily requirement of insulin varied between 20 and 50 U. per day. In two instances, a female of 36 and

another female of 24, chlorpropamide was used because of the brittle nature of the diabetes, and in the hope that it might facilitate the action of insulin. In both instances it failed in less than 1 week, 1 patient developing a severe ketosis that necessitated admission to hospital.

Four patients who had been inadequately controlled by diet and tolbutamide were transferred to chlorpropamide. One patient failed to respond. She was 80 years old and, earlier in her diabetic history, had been controlled with 56 U. of protamine zinc insulin each day. She had been transferred to 1.5 gm. tolbutamide per day, but showed no response and, likewise, was not influenced by chlorpropamide.

In this trial there have been no unexpected episodes either in response or in failure to respond to this new sulfonyl compound. The side effects observed

TABLE 3
DATA FOR THIRTEEN PATIENTS FAILING TO RESPOND TO TREATMENT

	Sex	Age	Wt.	Dose	Duration	Side effects	Duration of diabetes
Previously untreated	M	54	158	1.5	2 weeks	Nausea	1 year
	F	64	155	1.0	2 weeks	—	4 months
Diet alone	F	73	124	0.75	3 months	—	4 months
	M	65	145	1.5	1 month	Nausea	2 months
	F	65	143	0.75	2 months	—	6 years
Diet and tolbutamide	F	80	200	0.50	5 months	—	5 years
Diet and insulin	F	63	177	1.0	2 weeks	—	12 yrs. 40 NPH
	F	24	146	1.0	2 weeks	Ketosis	9 yrs. 20 NPH
	F	52	136	1.0	2 weeks	—	6 yrs. 24 NPH
	F	55	143	1.0	2 weeks	—	10 yrs. 24 R, 24 PZ
	F	74	86	3.0	1 week	Insulin allergy, nausea	20 yrs. 40 NPH
	F	72	150	0.5	2 weeks	Diarrhea	2 yrs. 28 R, 20 PZ
	F	36	136	0.75	1 week	Ketosis	20 yrs. 48 NPH

have been relatively insignificant. A small number of patients complained of nausea, and this was more apparent in those cases in which the dosage was raised to more than 0.5 or 0.75 gm. per day. In fact almost every case in which the dose was raised to more than 0.75 gm. daily there was some complaint of nausea or dizziness. In 2 instances a photosensitive rash, no different from that observed in other forms of sulfonamide therapy, occurred and, in both, this was of minor degree. On cessation of the drug the rash soon disappeared. In 2 instances albuminuria developed and the drug was discontinued despite the fact that there was no significant alteration in renal efficiency tests. It did not seem that any obvious renal damage had occurred. In all instances in which hepatic efficiency tests were performed there was neither clinical nor biochemical evidence that any hepatic disturbance was

occurring or had occurred as a result of chlorpropamide therapy. In no instance was any depression of blood formation observed. In three cases a curious effect was noted by patients after the consumption of preprandial alcoholic drinks; all complained of sensations of flushing of the face, which was obvious to onlookers and lasted for several hours.

In view of our previous experience that carbutamide and tolbutamide both apparently inhibit liver transaminase it was decided to investigate the effect of chlorpropamide and tolbutamide on the oxidation of C^{14} -labeled amino acids by liver slices, using the drugs at a more physiological concentration than in previous enzyme experiments.³

The slices were incubated in Krebs phosphate buffer at a glucose concentration of 100 mg. per 100 ml. and a C^{14} DL-alanine concentration of 40 mg.

TABLE 4
EFFECT OF TOLBUTAMIDE AND CHLORPROPAMIDE ON THE OXIDATION OF C^{14}
ALANINE BY LIVER SLICES

	No. Series	$C^{14}O_2$ recovered as cpm/mg. liver/hour	Difference	P
<i>Experiment 1</i>				
Control.....	6	108.5 ± 9.8 (S.E.M.)		
Chlorpropamide.....	6	154.7 ± 14.2	46.2	<0.01
<i>Experiment 2</i>				
Control.....	6	98.2 ± 3.4		
Tolbutamide.....	6	106.3 ± 3.4	8.1	<0.01

per 100 ml. Either chlorpropamide or tolbutamide was added to 1 of each pair of slices to give a final concentration of 30 mg. per 100 ml.

The $C^{14}O_2$ was collected on a KOH-soaked paper spill in the center well of the Warburg vessel used, then eluted and precipitated as $BaC^{14}O_3$; the radioactivity counted was corrected to infinite thinness. The results of this study are shown in TABLE 4. It is seen that both compounds accelerated the oxidation of the keto-acid moiety of the amino acid, in contradistinction to the result that might have been anticipated from the enzyme experiment.

Although these results appear at variance, it is likely that the apparent inhibition of transaminase may well have been due to accelerated oxidation of the acceptor α -ketoglutaric acid in the enzyme experiment, leading to a diminution in the amount of glutaric acid recovered. However, the suggestion that at least part of the action of these compounds is due to inhibition of neoglucogenesis is still valid, although the effect may be an acceleration of oxidation of Krebs-cycle intermediates rather than an inhibition of their formation. It is interesting to note that, at the same concentration, chlorpropamide is roughly five times as effective in promoting the oxidation of alanine as is tolbutamide.

To practicing physicians, the discovery of the hypoglycemic action of

the sulfonylureas has provided a fresh interest in the management of diabetic patients. For many years it has been thought desirable, if not essential, to control hyperglycemia and to reduce glycosuria. The application of these principles has produced a remarkable improvement in the life and welfare of diabetic patients. However, it has been obvious for some time that control of hyperglycemia does not prevent patients from developing peculiar complications that affect the vascular and nervous systems. This has led to the speculation that diabetes mellitus is not always a problem of insulin deficiency alone and that substitution therapy with insulin does not prevent the development of complications, at least in some patients. It is possible that physicians tend to forget that insulin has effects on the metabolism of proteins and of fats impossible to assess by any simple clinical means, and that they place too much emphasis on easily performed tests for glucose in blood and urine.

Consequently, when a chemical substance whose action is as yet unknown is found to be capable of reducing hyperglycemia in some patients and not in others it is not surprising that something resembling a therapeutic tumult has occurred. Eighty years ago Lancereaux⁵ pointed out the clinical difference between *diabete maigre* and *diabete gras*, a distinction emphasized by Hims-worth⁶ in 1949 and proved by Lawrence and Bornstein in 1950.⁷ It seems that the sulfonylurea compounds are capable only of influencing those patients who are still able to produce some insulin of their own; naturally, fears have arisen that they may be disturbing bodily functions in a harmful fashion. Certainly it seems possible, from the *in vitro* studies already described, that some deviation of normal metabolic processes occurs although, thus far, we have no evidence that this is either harmful or dangerous. If the diabetes of middle life is due to excessive neoglucogenesis it could be that these compounds alter or even correct such a deviation from normal metabolism. This could offer an explanation for those cases whose hyperglycemia, previously controlled with appreciable dosages of insulin, is capable of control with small amounts of these new drugs given each day.

From this brief experience it would seem that chlorpropamide is capable of controlling the hyperglycemia of an appreciable number of diabetic patients who have developed the complaint in middle age.

No harmful side effects have been observed thus far in any of the patients treated with chlorpropamide.

There appears to be no qualitative difference between the patients who respond to carbutamide and those responsive to tolbutamide or chlorpropamide.

There appears to be a significant difference between the required dosages of chlorpropamide and tolbutamide in the patients who show response to these drugs. This is also shown by *in vitro* studies on the effect of these drugs in the promotion of oxidation of alanine.

The suggestion is made that the action of these sulfonyl drugs is concerned with an acceleration of oxidation of Krebs-cycle intermediates and that an inhibition of neoglucogenesis might explain their influence on some types of human diabetes.

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THE EFFECT OF CHLORPROPAMIDE ON ALLOXAN DIABETES IN MICE*

Francisco Grande and Carlos Martínez

University of Minnesota, Minneapolis, Minn.

Diabetes has been produced in mice by Kirschbaum and Bell¹ after subcutaneous injection of alloxan at the rate of 200 mg./kg. body weight, and by Lazarow² after intravenous administration of 50 to 70 mg./kg. More recently we have reported³ the differences in the diabetogenic effect of intravenously injected alloxan on different strains of mice. The present paper describes some preliminary observations about the effect of chlorpropamide† on the alloxan diabetic mice.

Methods

Alloxan monohydrate (50 to 70 mg./kg. body weight) was injected into the tail vein of adult male ZCeF₁ hybrid mice as previously described.³ Blood samples taken by cutting the tip of the tail were analyzed for blood sugar by the Somogyi-Nelson method.⁴ Of twenty-eight animals injected, all developed marked hyperglycemia forty-eight hours after injection. Ten animals died during the two weeks following the injection of alloxan; four weeks thereafter, eight of the surviving animals were normoglycemic, while the other ten were still severely diabetic.

Chlorpropamide was given by stomach tube once a day at a dosage of 100 mg./kg. of body weight for 16 days. Blood samples were taken at 3 and 10 days after the administration of chlorpropamide, and at 2 and 9 days after chlorpropamide was discontinued. A group of 8 normal mice not injected with alloxan was used as control and treated with chlorpropamide simultaneously and in the same way.

Results

The results are summarized in TABLE 1, which gives the mean blood sugar values (and standard error of the means) for the different groups of animals. The blood sugar values of the control animals before chlorpropamide was given are in the same range as those reported by Lazarow.² The blood sugar values of these animals did not change after the administration of chlorpropamide for 3 days (column 6, TABLE 1). A small decrease of 27 mg./100 ml. was observed after 10 days of administration (column 7, TABLE 1), but this decrease proved to be not significant ($t = 1.45$; $p > 0.1$).

Similar response was observed in the group of animals with transient diabetes. The mean blood sugar value 10 days after chlorpropamide was 27 mg./100 ml. lower, but this decrease was statistically not significant ($t = 1.1$; $p > 0.1$).

* The study reported in this paper was aided by grants from the Minnesota Division of the American Cancer Society, Inc., New York, N. Y., and the Public Health Service, Bethesda, Md.

† Supplied by Chas. Pfizer & Co., Inc., Brooklyn, N. Y.

In both groups there were animals that showed very marked decrease in blood sugar; 1 animal in the control group had a decrease of 93 mg./100 ml. 10 days after administration of the chlorpropamide and 1 other, in the transient diabetes group, had a decrease of 135 mg./100 ml.

In the diabetic group the administration of chlorpropamide produced a very marked decrease of the blood sugar 3 days after the beginning of treatment (column 6, TABLE 1). The mean decrease was 178 mg./100 ml., or

TABLE 1
EFFECT OF CHLORPROPAMIDE IN MICE*

Group	Number of animals	Mean blood sugar value and S.E. of mean†						
		5/29	6/5	6/26	6/30	7/7	7/14	7/21
Transient diabetes.....	8	314 ±45	401 ±82	159 ±12	163 ±10	132 ±21	159 ±14	175 ±4
Permanent diabetes.....	10	383 ±36	561 ±52	334 ±11	156 ±19	165 ±4	183 ±7	234 ±24
Control.....	8	—	—	162 ±5	166 ±10	135 ±15	168 ±6	180 ±6

* Alloxan was injected on May 14. Administration of chlorpropamide (100 mg./kg.) began on June 27 and continued for 16 days through July 12.

† Upper figure of figure pair is mean blood sugar value in milligrams per 100 ml.; lower figure is standard error of the mean.

53 per cent of the value before chlorpropamide. All the animals had hypoglycemic response; the smallest individual blood sugar decrease observed was 90 mg./100 ml. A slight increase of the blood sugar was observed in this group 2 days after discontinuing administration of chlorpropamide, but the values had not returned to the pretreatment level after 9 days.

Comment

The results indicate a very marked hypoglycemic effect of chlorpropamide in the alloxan diabetic mice, which contrasts with the very slight effect observed in the control animals and in those that were injected with alloxan but that were nondiabetic at the time of the administration of chlorpropamide. These observations are at variance with other observations regarding the effect of the sulfonylureas on the alloxan diabetic animal.⁵⁻⁷ It would appear that chlorpropamide is more effective in decreasing the blood sugar of the diabetic mouse than in producing hypoglycemia in the normal animal.

Summary

Twenty-eight mice were injected intravenously with 50 to 70 mg. of alloxan. All the animals developed marked hyperglycemia. Four weeks after injection of alloxan, 8 of the animals were normoglycemic. Chlorpropamide was given to these animals by stomach tube at a dosage of 100 mg./kg. of

body weight, as well as to 10 others that were still diabetic, and to 8 normal controls. Three days after the administration of chlorpropamide, the alloxan diabetic mice showed a very marked decrease of their blood sugar level. No significant effect was produced in the other two groups of animals.

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THE EFFECTS OF TOLBUTAMIDE AND CHLORPROPAMIDE ON PATIENTS EXHIBITING JAUNDICE AS A RESULT OF PREVIOUS CHLORPROPAMIDE THERAPY

L. Harvey Hamff, Harold A. Ferris, Edwin C. Evans, Harold W. Whiteman
Emory University, Atlanta, Ga.

The control of diabetes mellitus with the oral sulfonylurea drugs in place of insulin has been accomplished in a large percentage of the total diabetic population. With the use of these agents one must be mindful of the possible undesirable side effects that occur. Jaundice as a complication of carbutamide therapy has been reported.^{1, 2} Recently jaundice has also been reported³ as a complication in tolbutamide therapy. Chlorpropamide is closely related to the above compounds, differing from them in having a chloride group substituted on the benzene ring, and a propyl instead of a butyl group at the end of the chain. With such a compound, one might anticipate the same type of allergic reactions as have occurred with carbutamide and tolbutamide. In studying twenty diabetic patients for their hypoglycemic effects of chlorpropamide, three cases of jaundice with associated nausea and vomiting and with an erythematous maculopapular rash were encountered. The following case reports are illustrative.

Case I. D. J., a 58-year-old Negro female, has had known diabetes mellitus since 1949 and was controlled on diet only until February 1958. Because of glycosuria, an elevated fasting blood sugar, and weight loss, therapy was begun with 10 U. of NPH insulin each morning before breakfast. Upon this regimen her diabetes became well controlled. On March 19, 1958, insulin was discontinued and chlorpropamide therapy was instituted with a dose of 0.5 gm. twice daily. The day before, a complete blood count was normal, with no eosinophilia present. Urinalysis was negative except for a 1+ sugar. Liver function tests were normal. The thymol turbidity test was 3.3 U., the alkaline phosphatase test was 3.78 Bodansky units, and the total serum bilirubin was 0.28 mg. per cent with a direct bilirubin of 0.13 mg. per cent.

Initially, the patient tolerated the medication satisfactorily and was managed with the same degree of control as with the previous insulin regimen. During the third week of chlorpropamide therapy she developed anorexia, nausea and vomiting, a dark urine, pruritus, and a skin rash. She stopped her medication on April 12, 1958. She was seen in the diabetes clinic on April 18 with the above complaints, and was admitted to the Medical Service at Grady Memorial Hospital. Careful questioning revealed no history of exposure to rats or hepatotoxic agents, or to anyone with hepatitis. Needle punctures were limited to her own insulin syringe and needle, used by no one else, and to those done in our laboratory with careful precautions.

Upon examination, the patient appeared acutely ill; her temperature was 103° orally, the pulse rate was 116/min., and blood pressure was 160/80. A generalized maculopapular rash spared only the palms and soles. There

were no mucous membrane lesions, the sclerae were icteric, and there were grade-2 arteriosclerotic changes of the fundi. The lungs were clear. The heart was slightly enlarged to the left; a grade-2 apical systolic murmur was recorded. The abdominal examination revealed no evidence of hepatomegaly. No organs or masses were palpated. The remainder of the examination was noncontributory.

When the patient was admitted, the blood count showed a Hb of 12.4 gm.; Hct, 41; and WBC, 3900, with 14 per cent bands, 57 per cent neutrophils, 6 per cent eosinophils, 17 per cent lymphocytes, and 6 per cent monocytes. Platelets were adequate. By April 30 the Hb had dropped to 10.5 gm. and the Hct to 32.5. WBC was 9400, with 13 per cent eosinophils, 46 per cent neutrophils, 2 per cent bands, 6 per cent metamyelocytes, 31 per cent lymphocytes, and 2 per cent monocytes. By May 27 the blood picture had returned to normal, revealing a Hb of 11.8 gm.; Hct, 35; and WBC, 6700, with 4 per cent eosinophils, 56 per cent neutrophils, 4 per cent bands, and 36 per cent lymphocytes.

Urinalysis on admission of the patient revealed a trace of sugar, 4+ acetone, and 4+ bile. The CO_2 -combining power was 23.9 mmoles/l. The 4+ acetone was thought to have been determined on the basis of starvation. Liver function tests on April 18, 1958 revealed a total protein of 7.1 gm.; albumin, 3.9 gm.; and globulin, 3.2 gm., with an albumin-globulin (A/G) ratio of 1.22. The thymol turbidity test was 2.25 U., and the cephalin flocculation test was 1+. The alkaline phosphatase test was markedly elevated to 26.9 Bodansky units. The serum bilirubin was elevated to 10.7 mg. per cent with a direct bilirubin of 7.65 mg. per cent. The prothrombin time of the patient was 43.4 sec., with a control value of 11.4 sec. at 100 per cent; 19.8 sec. at 30 per cent; and 26 sec. at 20 per cent.

After the intravenous administration of 50 mg. of vitamin K_1 , the prothrombin time of the patient on April 19 was 14.0 sec., with a control value of 12.4 sec. at 100 per cent; 21 sec. at 30 per cent; and 27.6 sec. at 20 per cent. On April 21 the prothrombin time was 15.0 sec., with the same control values as on April 19. On April 28 it was 13.0 sec., with a control value of 13.4 sec. at 100 per cent; 21.0 sec. at 30 per cent; and 29.0 sec. at 20 per cent. Serum electrophoresis on April 25 revealed a nonspecific elevation of the serum globulins.

By April 24 the alkaline phosphatase had dropped to 16.1 Bodansky units. After the patient was discharged from the hospital, the alkaline phosphatase was 5.66 Bodansky units on May 20, 4.39 on May 27, 6.88 on June 3, and 3.84 on June 10, 1958. Since the last date these values have remained normal.

On April 21 the total serum bilirubin was 8.5 mg. per cent, with a direct bilirubin of 6.1 mg. per cent. On April 22 the total serum bilirubin was 10.9 mg. per cent, with a direct bilirubin of 7.9 mg. per cent. On April 28 total serum bilirubin was 6.3 mg. per cent, with a direct bilirubin of 5.0 mg. per cent. After the patient was discharged from the hospital on May 20, the total serum bilirubin was 2.45 mg. per cent, with a direct bilirubin of 1.6 mg. per cent. On May 27 the total serum bilirubin was 1.5 mg. per cent,

with a direct bilirubin of 1.22 mg. per cent. On June 3, the total serum bilirubin was 0.90 mg. per cent, with a direct bilirubin of 0.80 mg. per cent. Since the last date the serum bilirubin values have remained within a normal range.

While the patient was in the hospital and since her discharge, the total proteins, albumin-globulin ratios, cephalin flocculation tests, and thymol turbidity tests have been within normal limits.

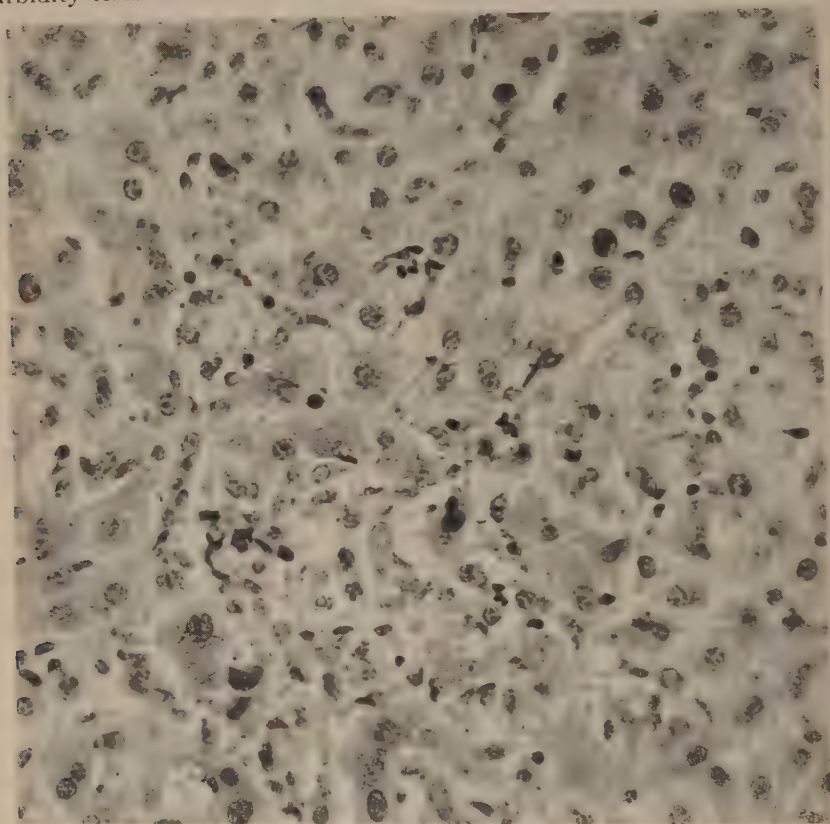


FIGURE 1. Hepatocellular damage as revealed by biopsy. $\times 160$.

A liver biopsy was obtained on April 22, 1958. FIGURE 1 shows the microscopic changes that took place; there was a marked increase in mitotic activity and abnormal cell forms as a result of a distorted growth pattern. Hepatocellular, intracanalicular, and littoral cell jaundice were present. Rare eosinophilic granulocytes were noted. Stasis of bile in the smaller canaliculi was present. The changes were those of cholestasis and hepatocellular damage. A chest X ray was suggestive of slight cardiomegaly, but was otherwise negative.

For the first 4 days in the hospital a daily temperature elevation as high as 102.2° to 103.8° occurred. From the sixth through the eighth day the

temperature did not exceed 100° orally, and the patient remained afebrile for the remainder of her hospital stay. For the first 2 days she received parenteral fluids and insulin. By the third day she was placed on a 1200-Cal. diet of 125 gm. carbohydrate, 60 gm. protein, and 40 gm. fat, which was increased to 1800 Cal. as 180 gm. carbohydrate, 80 gm. protein, and 84 gm. fat when her appetite returned to normal. A fasting blood sugar was 95 mg. per cent on April 22. With diet and 10 U. of NPH insulin each morning before breakfast she remained aglycosuric and was discharged as improved on this regimen on May 7, 1958.

After her complete recovery, insulin was discontinued and tolbutamide therapy was begun on June 21, the patient receiving 3.0 gm. the first day, 2.0 gm. the second day, and 1.0 gm. daily thereafter. She remained asymptomatic. Weekly blood counts, urinalyses, thymol turbidity tests, cephalin flocculation tests, A/G ratios, alkaline phosphatase tests, and total and direct serum bilirubin values remained normal. Tolbutamide was continued for 7 weeks until August 9, 1958, when chlorpropamide was substituted at a dosage of 250 mg. daily. On this reduced dosage of chlorpropamide she has remained asymptomatic and aglycosuric, with normal blood glucose levels for the 5 weeks she has been on the drug. Weekly blood counts, urinalyses, thymol turbidity tests, cephalin flocculation tests, A/G ratios, alkaline phosphatase tests, and total and direct serum bilirubin values have been determined and have been found to remain within normal limits.

Case 2. O. D., a 68-year-old Negro female was discovered to have diabetes mellitus in December 1955, with the typical symptoms of polyuria, polydipsia, polyphagia, a 15-lb. weight loss, lack of energy, and pruritus vulvae for 8 months. Her diabetes was treated with diet and 30 U. of NPH insulin each morning before breakfast. On October 3, 1956 she was admitted to the Surgical Service of Grady Memorial Hospital for cholecystectomy. At the age of 26 years she had had typhoid fever followed by jaundice. In April 1956 the patient developed right upper quadrant pain and associated nausea and vomiting. Cholecystogram at this time revealed nonopacification of the gall bladder and cholelithiasis. Because of continued symptoms of indigestion and intermittent attacks of upper abdominal discomfort with radiation into the right scapular region, cholecystectomy was performed. Although her sclerae were thought to be slightly icteric, on October 4, 1956 the total serum bilirubin was 0.3 mg. per cent, with a direct bilirubin of 0.2 mg. per cent. At the same time the total protein was 6.4 gm.; albumin, 3.1 gm.; and globulin, 3.3 gm. The A/G ratio was 0.94, thymol turbidity was 4 U., and the cephalin flocculation test was 3+. These tests were repeated on October 5, 1956. The total serum protein was 7.6 gm.; albumin, 3.1 gm.; and globulin, 4.5 gm. The A/G ratio was 0.69, thymol turbidity was 4 U., and the cephalin flocculation test was 2+. The patient withstood cholecystectomy uneventfully and was discharged from the hospital October 18, 1956 on an 1800-Cal. diet of 175 gm. carbohydrate, 70 gm. protein, and 91 gm. fat, with 35 U. of NPH insulin each morning before breakfast. Her case was followed at the diabetes clinic and, on January 4, 1957, the NPH

insulin dosage was reduced to 30 U. because of occasional mild hypoglycemic reactions. She remained only fairly well controlled on this regimen, intermittently showing glycosuria. On April 2, 1958, insulin was discontinued and chlorpropamide therapy was instituted with a dose of 0.5 gm. twice daily. The day before, a complete blood count was normal, urinalysis was negative (except for a 4+ sugar), liver function tests were normal, the thymol turbidity test was 4.3 U., the alkaline phosphatase test was 3.27 Bodansky units, and the total serum bilirubin was 0.35 mg. per cent, with a direct bilirubin of 0.15 mg. per cent.

Initially, the patient tolerated the medication satisfactorily and was managed with the same degree of control as with the previous insulin regimen. On the fourteenth day of chlorpropamide therapy the patient developed nausea and vomiting and a dark urine that persisted for 3 days (until April 18, 1958) when she was admitted to the hospital. Careful questioning revealed no history of exposure to rats or hepatotoxic agents, or to anyone with hepatitis. Needle punctures were limited to her own insulin syringe and needle, used by no one else, and to those done in our laboratory with careful precautions.

Examination showed the patient to be in no acute distress; her temperature was 99° orally, the pulse rate was 88/min., and the blood pressure was 144/80. A faint maculopapular rash was present over the trunk, the sclerae were icteric, the lungs were clear, and the heart sounds were normal. Abdominal examination revealed a cholecystectomy scar. The liver edge was felt two finger breadths below the right costal margin and was nontender. The remainder of the examination was noncontributory. The urinalysis on admission was negative except for the presence of bile; the blood count was within normal limits. Liver function tests on April 18, 1958 revealed a total protein of 7.3 gm.; albumin, 3.4 gm.; globulin, 3.9 gm.; and an A/G ratio of 0.87. The thymol turbidity test was 4.2 U. and the cephalin flocculation test was 1+. The alkaline phosphatase test was elevated to 8.5 Bodansky units. The serum bilirubin was elevated to 6.35 mg. per cent, with a direct bilirubin of 4.25 mg. per cent.

The prothrombin time of the patient was 39.2 sec., with a control value of 11.4 sec. at 100 per cent, 19.8 sec. at 30 per cent, and 26.0 sec. at 20 per cent. Following the intravenous administration of 50 mg. of vitamin K₁, the prothrombin time on April 19 was 15.0 sec. with a control value of 12.4 sec. at 100 per cent, 21.0 sec. at 30 per cent, and 27.6 sec. at 20 per cent. On April 21, it was 14.2 sec., with the same control values as on April 19. On April 28 it was 13.4 sec. with a control value of 13.4 sec. at 100 per cent, 21.0 sec. at 30 per cent, and 29.0 sec. at 20 per cent. On April 22, 1958 the total serum bilirubin had dropped to 1.5 mg. per cent, with a direct bilirubin of 0.9 mg. per cent. On April 28 the total serum bilirubin was 1.15 mg. per cent, with a direct bilirubin of 0.63 mg. per cent. Since then, weekly bilirubin values have remained normal.

The alkaline phosphatase test was not repeated until May 26, 1958, when it was 2.8 Bodansky units. Weekly determinations since then have remained normal.

On April 22, 1958 there was an increase in reversal of the A/G ratio, with a total protein of 7.2 gm.; albumin, 3.1 gm.; globulin, 4.1 gm., and an A/G ratio of 0.76. On April 25 serum electrophoresis revealed a nonspecific increase in globulins. On April 28 the total protein was 7.7 gm.; albumin, 4.5 gm.; globulin, 3.2 gm.; and an A/G ratio of 1.4. Weekly A/G ratios have remained normal. A Bromsulphalein test on April 28, revealed 10 per cent retention of dye after 45 min. This was repeated on July 15, 1958, with a retention of 4 per cent.

A liver biopsy was obtained on April 23, 1958 after the patient had almost completely recovered. The biopsy revealed minimal hepatocellular degeneration, minimal parenchymal cell jaundice, and minimal pericholangitis with bile stasis.

A chest X ray was negative. While in the hospital the patient remained afebrile. By the following day the nausea and vomiting had subsided, and a sense of well-being returned. The diabetic state was well controlled on an 1800-Cal. diet of 175 gm. carbohydrate, 70 gm. protein, 91 gm. fat, and 30 U. of NPH insulin each morning before breakfast. She was discharged without complaints on April 28, 1958.

One month after complete recovery insulin was discontinued; tolbutamide therapy was begun on June 1, 1958 in dosages of 3.0 gm. the first day, 2.0 gm. the second day, and 1.0 gm. daily thereafter for 7 weeks. Weekly blood counts, urinalyses, thymol turbidity tests, cephalin flocculation tests, A/G ratios, alkaline phosphatase tests, and total and direct serum bilirubin values have remained normal. The cephalin flocculation test was 3+ on 2 occasions, but negative to 2+ in the remaining tests. She remained asymptomatic and fairly well controlled on this regimen. On July 19, 1958 tolbutamide was discontinued and chlorpropamide was begun with a daily dosage of 250 mg. For 8 weeks she has remained asymptomatic, and is now essentially aglycosuric. On August 28, 1958 the fasting blood sugar was 137.5 mg. per cent, and a blood sugar 2 hours after breakfast was 175 mg. per cent. Weekly blood counts, urinalyses, cephalin flocculation tests, and total and direct serum bilirubin values were normal.

Case 3. L. C., a 50-year-old Negro female, was seen at the eye clinic in December 1955, complaining of difficulty with vision in the left eye. She was found to have bilateral cataracts. When she was admitted on January 9, 1956 for cataract extraction, glycosuria was found and the fasting blood sugar was 282 mg. per cent. Repeat fasting blood sugars were 313 mg. per cent and 264 mg. per cent. On a 1500-calorie diet of 160 gm. carbohydrate, 55 gm. protein, and 70 gm. fat, she developed ketosis before insulin treatment was started. After cataract extraction was accomplished on the left eye she was discharged February 6, 1956, and placed on a 1500-Cal. diet with 20 U. of NPH insulin each morning. On April 13, 1956 she was seen in the diabetes clinic. At this time, her weight was 95½ lb., and the urine was negative for sugar. Since the patient had experienced a few episodes of mild hypoglycemia, the insulin dosage was reduced to 15 U. of NPH with the continuation of a 1500-Cal. diet. She maintained fairly good control of her diabetes on this regimen, with occasional glycosuria. On January 16, 1957 cataract

extraction was performed on the right eye. The patient was observed at occasional intervals and seemed to be under fairly good control with 15 U. of NPH insulin each morning; the weight varied usually between 100 and 105 lb. On March 18, 1958 insulin was discontinued and the patient was started on 0.5 gm. chlorpropamide twice daily. At this time the total serum bilirubin was 0.25 mg. per cent, with a direct bilirubin of 0.14 mg. per cent; the thymol turbidity was 2 U. and the alkaline phosphatase was 4.87 Bodansky units. On March 28 the patient was aglycosuric and was feeling well; her weight was 105 lb. On April 18, 1958 anorexia, nausea, vomiting and generalized pruritus appeared and dark urine was noted for 2 or 3 days. Chlorpropamide was discontinued and 15 U. NPH insulin each morning was again started on April 21, 1958. On April 25, 1958 she stated that the gastrointestinal symptoms disappeared as soon as she stopped taking chlorpropamide and the urine became normal in color about two days later; there was still some pruritus and her weight was 98 lb. The sclerae were mildly icteric and there was an erythematous maculopapular rash on the trunk. The liver and spleen were not definitely palpable. Careful questioning revealed no history of exposure to rats or hepatotoxic agents, or to anyone with hepatitis. Needle punctures were limited to her own insulin syringe and needle, used by no one else, and to those done in our laboratory with careful precautions. On April 28 the total serum bilirubin was 1.85 mg. per cent, with a direct bilirubin of 0.9 mg. per cent. The thymol turbidity test was 1.45 U., alkaline phosphatase test was 13.2 Bodansky units, cephalin flocculation test was 1+, and A/G ratio was 0.92. The prothrombin time was normal. All symptoms and signs cleared rapidly so that on April 30, 1958 she appeared well, except for slight scaling of the skin over the areas of the abdomen previously involved by the rash. There was occasional glycosuria on 15 U. of NPH insulin daily and the weight was 98 lb. On May 30 the thymol turbidity test was 3.7 U., cephalin flocculation test was 2+, alkaline phosphatase test was 10.94 Bodansky units, and the A/G ratio was 1.54:1. On May 31, 1958 insulin was discontinued and tolbutamide was started in a dosage of 3.0 gm. the first day, 2.0 gm. on the second day, and 1.0 gm. daily thereafter. The patient continued to do well, maintained her weight, and had no glycosuria or symptoms of hypoglycemia. On July 18 the total serum bilirubin was 0.35 mg. per cent, with a direct bilirubin of 0.20 mg. per cent, the thymol turbidity test was 2.0 U., the alkaline phosphatase test was 6.25 Bodansky units, the A/G ratio was 1.48:1, the cephalin flocculation test was 1+, and the BSP test showed 2 per cent retention of the dye in 45 min. On July 19, tolbutamide was discontinued and chlorpropamide was again started in a dosage of 250 mg. daily. The patient continued without glycosuria and felt well until July 27, 1958, when she developed nausea and vomited once. The patient immediately stopped taking chlorpropamide and resumed tolbutamide, 1.0 gm. each morning. On August 1, 1958 she was feeling well and showed no clinical evidence of jaundice, skin rash, or enlargement or tenderness of the liver. On this date the total serum bilirubin was 0.20 mg. per cent, with a direct bilirubin of 0.13 mg. per cent. The thymol turbidity test was 2.2 U., alkaline phosphatase test was 4.68

Bodansky units, A/G ratio was 1.41:1, cephalin flocculation was 1+, prothrombin time was normal, and the BSP test showed 2 per cent retention of the dye in 45 min. Since that time the patient has continued taking tolbutamide and has continued to feel well with good control of her diabetes. Weekly blood counts, urinalyses, thymol turbidity tests, cephalin flocculation tests, A/G ratios, alkaline phosphatase tests, and total and direct serum bilirubin values have remained normal.

Discussion

Although we can not rule out absolutely the coexistence of a viral hepatitis as the cause of the jaundice, the presence of an erythematous, maculopapular rash in all 3 patients and the presence of a 13 per cent eosinophilia in 1 patient suggest very strongly that the etiology of the jaundice was related to a hypersensitivity reaction to chlorpropamide. Many drugs have been stigmatized as producing jaundice. Probably the drug that now most commonly causes jaundice is chlorpromazine. J. H. Moyer *et al.*⁴ reported 1 case of obstructive jaundice without hepatocellular involvement in 500 patients treated. L. E. Hollister⁵ reported 17 cases of jaundice due to chlorpromazine among a total of more than 900 patients treated with the drug. He estimates that the over-all incidence of jaundice in patients treated for at least 1 week with chlorpromazine is probably between 1 and 2 per cent. The work of these investigators strongly suggests a hypersensitivity reaction as the cause of the hepatic complications. The clinical picture of fever, gastrointestinal symptoms, malaise, and jaundice developing within 4 to 5 weeks of therapy, together with the laboratory findings primarily of an obstructive type of jaundice, is very similar to the findings in our cases.

Methyltestosterone as a cause of jaundice has also been described.⁶ A. B. Gutman⁷ has discussed the drug reactions characterized by cholestasis associated with intrahepatic biliary tract obstruction. He reported the following drugs as causative agents: arsphenamine, dinitrophenol, cinchophen, aurothioglucose, methyltestosterone, norethandrolone (Nilevar), thiouracil, para-aminosalicylic acid, sulfadiazine, methimazole, 8-(para-aminobenzyl) caffeine. He considered these reactions to be the result of hypersensitivity to the drugs involved. F. A. Gibbs and F. W. Stamps⁸ report serious liver damage occurring in about four persons in one thousand receiving phenurone (phenacetylcarbamide) as an anticonvulsant. These effects also should be considered as hypersensitivity reactions.

Chlorpropamide, as well as carbutamide and tolbutamide, is a sulfonamide derivative. Hepatotoxic changes with jaundice as a result of sulfonamides used as chemotherapeutic agents have been well known for some time.^{9, 10} It is thought that a hypersensitivity reaction is responsible for these side effects, although the exact mechanism of action on the liver is not definitely known.

The predominant picture in our cases was an obstructive jaundice with hyperbilirubinemia, an elevated alkaline phosphatase, and only slight abnormalities in hepatocellular function. It was interesting that two of the

patients had a marked elevation of the prothrombin time. Another evidence that serious hepatocellular damage had not occurred was the prompt return to normal of the prothrombin time after vitamin K₁ therapy. Cholestasis was a prominent feature of the liver biopsy findings, although hepatitis was also present. The similarity of our findings to the reports of hypersensitivity reactions with jaundice due to various drugs makes us feel that our cases definitely have this basis.

Because of a rather high incidence of cross sensitivity to the sulfonamides, we attempted to determine whether these patients would show any side effects when placed on tolbutamide therapy. With an initial dose of 3.0 gm., a second dose of 2.0 gm., and a daily dose of 1.0 gm., there was no evidence of hypersensitivity. The patients remained asymptomatic, and weekly blood counts, urinalyses, and liver function tests remained normal for the 7 weeks they were kept on tolbutamide.

After tolbutamide therapy, these patients were again placed on chlorpropamide, but with a reduction of the dose from 1.0 gm. to 250 mg. daily. Two of the 3 have tolerated the drug without side effects for 5 and 8 weeks, respectively. They have remained asymptomatic. Weekly blood counts, urinalyses, and liver function tests have remained normal. On the ninth day of resumption of chlorpropamide, the third patient noticed nausea and vomited once. She immediately discontinued the drug on her own volition and resumed tolbutamide, which she has continued to take without incident. At the time she stopped chlorpropamide no abnormalities by physical examination, blood count, urinalysis, or liver function tests could be found. We are not in a position to state whether these gastrointestinal complaints were related to the drug or were due to some unrelated cause. One might expect evidence of hypersensitivity when the patients were again administered the drug. In 1 of these patients it may have been the cause of the gastrointestinal complaints. Possibly the reduction in dosage may have decreased the likelihood of this reaction. With true hypersensitivity, however, one would have expected some evidence of hypersensitivity even with a smaller dose of the drug. L. E. Hollister⁵ reported recurrence of jaundice in 6 of 11 patients treated with chlorpromazine, 9 of the 11 revealing some evidence of hypersensitivity. None of his patients showed cross sensitivity to chlorpromazine.

Summary

Three cases of jaundice as a hypersensitivity reaction to chlorpropamide have been presented.

The jaundice was primarily an obstructive type, with hyperbilirubinemia and an elevated alkaline phosphatase.

There was no evidence of cross sensitivity to tolbutamide.

No recurrence of jaundice or evidence of impairment of hepatic function occurred when a smaller dose of chlorpropamide was given.

On the ninth day of resumption of chlorpropamide, one case developed mild gastrointestinal symptoms. These symptoms cannot be definitely related to the drug.

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STUDIES ON THE USE OF CHLORPROPAMIDE IN PATIENTS WITH DIABETES MELLITUS*

N. Sheldon Skinner Jr.,† Robert L. Hayes,‡
S. Richardson Hill, Jr.

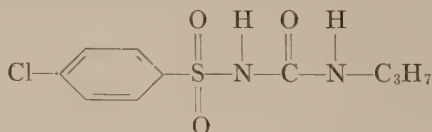
Department of Medicine and University Hospital, The University of Alabama Medical Center, and the Medical Service, Veterans Administration Hospital, Birmingham, Ala.

The study of oral hypoglycemic agents has greatly stimulated research in diabetes mellitus. Many new compounds capable of producing hypoglycemia are being synthesized and studied in an effort to find one such compound that will prove to be as effective as insulin in controlling diabetes mellitus and yet still be suited to oral administration.

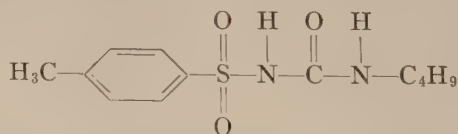
It is the purpose of this report to describe our clinical experience with chlorpropamide, a halogenated sulfonylurea compound, and to compare in detail the efficiency of this drug with that of insulin in managing patients with diabetes mellitus.

Chemistry and Pharmacology

Chlorpropamide has the chemical name of 1-propyl-3-(*p*-chlorobenzene-sulfonyl)urea and the chemical structure:



Tolbutamide, in comparison, has the chemical name of 1-butyl-3-tolyl-sulfonylurea and the chemical structure:



The LD₅₀ in mice and rats is comparable with both chlorpropamide and tolbutamide according to one study, while another group reports that chlorpropamide shows greater toxicity. Both of these compounds showed similar side effects (depression, ataxia, tremors) in mice and rats, usually only at the higher dose levels.^{1, 2}

Acute, subacute, and chronic toxicity studies of this drug done on mongrel

* The study reported here was supported in part by funds from the National Institute of Arthritis and Metabolic Diseases, Public Health Service, Bethesda, Md., and Chas. Pfizer & Co., Inc., Brooklyn, N. Y.

† Student Trainee in diabetes mellitus, National Institute of Arthritis and Metabolic Diseases, Public Health Service, Bethesda, Md.

‡ Student Research Fellow, The National Foundation, New York, N. Y.

dogs showed no significant anatomical changes, either gross or histological, according to one group of investigators, whereas a second group found liver degeneration and fatty infiltration as well as nephrotic changes in the kidney.^{1, 2}

Blood level determinations were done on normal human volunteers. Three groups of these volunteers were used; equal numbers of volunteers were placed on dose levels of 0.25 gm. per day, 0.50 gm. per day, and 1.0 gm. per day of chlorpropamide. Each of these groups was observed to reach a mean plateau blood level of chlorpropamide on approximately the fifth day of drug ingestion. The volunteers receiving 0.25 gm. per day reached plateau blood levels of approximately 10 mg. per cent; those receiving 0.50 gm. daily, approximately 16 mg. per cent; and those receiving 1.0 gm. daily, approximately 25 to 30 mg. per cent.¹

Three of the volunteers taking 1.0 gm. per day of chlorpropamide became hypoglycemic, thereby necessitating discontinuance of the compound. Blood levels were determined in these three after drug withdrawal, and it was found that the removal of chlorpropamide from the blood stream was accomplished very slowly, suggesting that no marked active destruction of this compound occurs in the human body, and that loss from the blood stream is accomplished by very slow renal excretion.

Materials and Methods

Fifty-two patients ranging in age from 25 to 88 years and with proved diabetes mellitus were maintained on chlorpropamide for periods ranging from 1 to 25 weeks. Forty-eight patients were observed at weekly or bimonthly intervals, depending on the need for a visit.

Four patients were carefully studied in the hospital on calculated diets. Four others had been on no previous treatment prior to the administration of chlorpropamide. Forty patients had been previously managed with insulin therapy, five with tolbutamide, and four by diet alone. Only two patients had been treated for diabetic acidosis prior to administration of chlorpropamide.

The selection of patients for the study was random, although in some instances patients were begun on chlorpropamide because of frequent insulin reactions, poor control with insulin, inability to self-administer insulin, or insufficient funds with which to purchase insulin (FIGURE 1).

All patients were placed on calculated diabetic diets, but only those who were evaluated during hospital admission had careful control of diet. The clinic patients, who constituted the lower economic stratum of the patients evaluated, did not observe rigid control of their diets.

Clinic patients were evaluated for degree of control with chlorpropamide therapy on the basis of fasting blood sugar examinations and urine examinations. The patients who were hospitalized were evaluated on the basis of fasting blood sugars, two- and three-hour postprandial blood sugars, and urine examinations. All blood sugars were determined by the Folin-Wu method.³

Twenty-three patients had serum protein-bound iodine (PBI) determina-

tions made before or within seven days following the administration of the initial dose of chlorpropamide; repeat PBI determinations were continued throughout the treatment period. The PBI determinations were made by a modification of the alkaline ash method of Barker.⁴

Twenty-two patients had serum cholesterol determinations made before or within seven days following the administration of the initial dose of

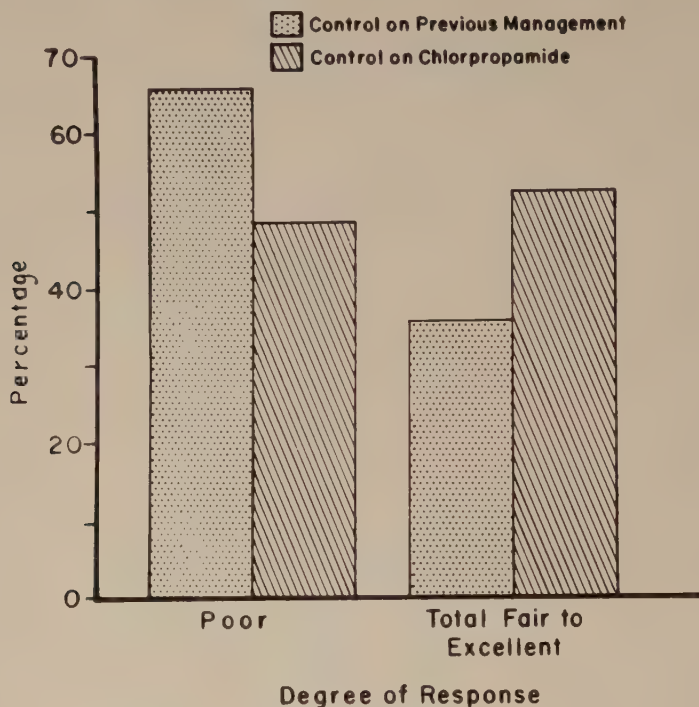


FIGURE 1. Comparison of the control of diabetes by chlorpropamide with previous management.

chlorpropamide. These determinations were made using the method of Pearson.⁵

All patients had weekly to bimonthly urinalyses, testing for red and white cells, albumin, specific gravity, sugar, and acetone. Three patients had repeated liver function tests using standard methods; that is, alkaline phosphatase, cephalin flocculation, and thymol turbidity. Four patients were evaluated with BSP tests. All patients were observed with total and differential white blood cell counts as well as packed-cell volumes or hemoglobin determinations.

Initially, all patients were begun on 2 gm. of chlorpropamide daily. The dose level was then quickly decreased to 1.0 or 0.5 gm. per day. The maintenance dose in the majority of patients treated was 0.5 or 0.25 gm. per day and never exceeded 1.0 gm. per day for any patient. The various degrees of control of the diabetes mellitus in each patient both before and after chlor-

propamide was administered were designated as follows: excellent, fasting blood sugar of 120 mg. per cent or less; good, 120 to 140 mg. per cent; fair, 140 to 160 mg. per cent; and poor, more than 160 mg. per cent.

Results

Blood sugar control. The effectiveness of chlorpropamide therapy in controlling diabetes mellitus was evaluated on the basis of the reduction in blood sugar level. The degree of blood sugar control was evaluated according to the age of onset of the diabetes, the amount of insulin required prior to

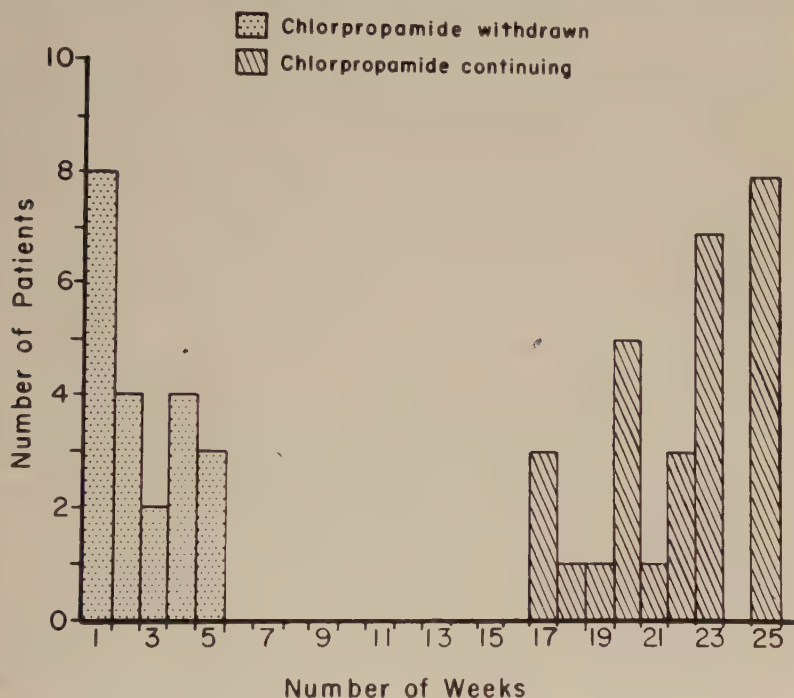


FIGURE 2. Duration of therapy with chlorpropamide.

treatment with chlorpropamide, the duration of diabetes prior to chlorpropamide treatment, and the age at which chlorpropamide treatment was begun.

FIGURE 2 shows the duration of chlorpropamide treatment in all patients studied. Of 52 patients begun on chlorpropamide, 22 (42 per cent) were stopped by the end of 5 weeks of treatment, and 30 (58 per cent) were maintained on treatment for 17 to 25 weeks. It should be noted that no patient was forced to withdraw from chlorpropamide therapy after 5 weeks of treatment. Of the 22 patients forced to stop chlorpropamide treatment, 6 had poor control of blood sugar without significant side reactions, 13 had poor control of blood sugar with significant side reactions, and 3 had fair-to-excellent control with significant side reactions.

FIGURE 1 shows graphically the poor and the fair-to-excellent control

attained on the various means of prechlorpropamide treatment and on chlorpropamide treatment.

TABLE 1 shows the degree of control of diabetes attained with chlorpropamide after 25 weeks of treatment. The over-all control was fair-to-excellent in 54 per cent and poor in 46 per cent of patients. While receiving various

TABLE 1
DEGREE OF CONTROL OF DIABETES OBTAINED WITH CHLORPROPAMIDE AFTER 25 WEEKS OF TREATMENT

Age groups	Control				
	Excellent	Good	Fair	Poor	Total
21 to 30.....				1	1
31 to 40.....				2	2
41 to 50.....	2	1	1	3	7
51 to 60.....	6	2	1	4	13
61 to 70.....	7	1	4	8	20
71 to 80.....	1	2		5	8
81 to 90.....				1	1
Total.....	16	6	6	24	52
Per cent total....	31.0	11.5	11.5	46	

TABLE 2
CORRELATION OF BLOOD SUGAR CONTROL WITH THE AGE OF ONSET OF DIABETUS MELLITUS

Age group (years)	Number of patients	Number of patients showing fair to excellent blood sugar response	Percentage of response
11 to 20.....	1	0	0
21 to 30.....	3	0	0
31 to 40.....	4	1	25
41 to 50.....	11	8	73
51 to 60.....	15	9	60
61 to 70.....	13	9	69
71 to 80.....	5	1	20

means of treatment prior to chlorpropamide administration, only 34 per cent of the 52 patients showed a fair-to-excellent response.

TABLE 2 shows a correlation of blood sugar control with the age of onset of diabetes mellitus. The 51 to 60 and 61 to 70 age groups had approximately the same percentage of fair-to-excellent responses, with the 41 to 50 age group showing the best response.

TABLE 3 shows a correlation of blood sugar response with the duration of

diabetes prior to chlorpropamide treatment. There is essentially an equal number of patients in each group up to the 15-year duration group. Because of the small number in the groups after this, no accurate evaluation can be made. The 0- to 1-year and 1- to 5-year duration groups showed, in effect, an equal degree of control, as did the 6- to 10- and 11- to 15-year duration groups.

TABLE 3
CORRELATION OF BLOOD SUGAR RESPONSE WITH THE DURATION OF DIABETES
PRIOR TO CHLORPROPAMIDE TREATMENT

Duration of diabetes (years)	Number of patients	Number of patients showing fair to excellent blood sugar response	Percentage of patients responding
Less than 1.....	16	9	54
1 to 5.....	14	10	72
6 to 10.....	9	2	22
11 to 15.....	10	5	50
15 to 20.....	1	1	100
20 to 25.....	1	1	100
30 to 35.....	1	0	0

TABLE 4
CORRELATION OF BLOOD SUGAR RESPONSE WITH AGES AT WHICH CHLORPROPAMIDE
TREATMENT WAS BEGUN

Age group (years)	Number of patients	Number of patients showing fair to excellent blood sugar response	Percentage of response
21 to 30.....	1	0	0
31 to 40.....	2	0	0
41 to 50.....	7	4	57
51 to 60.....	13	9	70
61 to 70.....	20	12	60
71 to 80.....	8	3	38
81 to 90.....	1	0	0

TABLE 4 shows a correlation of blood sugar response with the ages at which chlorpropamide treatment was begun. Four of the age groups shown have approximately the same number of patients, and all have essentially the same blood sugar response, with a fair-to-excellent response ranging from 38 to 70 per cent.

TABLE 5 shows a comparison of blood sugar control while on chlorpropamide with that obtained on previous therapy. Again, there is a general tendency that suggests that the lower the previously required insulin dose, the better was the response to chlorpropamide. A 100 per cent response to

chlorpropamide was noted in the five patients previously controlled on tolbutamide.

One hospitalized patient was carefully studied with both chlorpropamide and tolbutamide administration. During the testing of chlorpropamide the patient was observed with PBI determinations; cholesterol determinations; fasting, 11 A.M., 2 P.M., and 4 P.M. blood sugars; radioactive iodine¹³¹ (RAI) uptakes; and liver function tests. This patient was an obese 65-year-old Negro male with a history of diabetes mellitus of 20 years' duration. He had been well controlled on 35 U. of NPH U-80 insulin per day when he was first discovered to have diabetes mellitus. He was then maintained on doses

TABLE 5
COMPARISON OF BLOOD SUGAR CONTROL WHILE ON CHLORPROPAMIDE WITH THAT OBTAINED ON PREVIOUS THERAPY

Previous means of treatment	Number of patients	Number of patients showing fair to excellent blood sugar response	Percentage of patients responding
No insulin.....	9	4	45
1 to 20 U. insulin.....	9	6	67
21 to 50 U. insulin.....	13	7	54
51 to 100 U. insulin.....	13	5	40
101 to 200 U. insulin.....	2	1	50
Over 200 U. insulin.....	1	0	0
Tolbutamide.....	5	5	100

of tolbutamide ranging from 1 to 4 gm. per day, although excellent control required 4 gm. daily.

FIGURE 3 shows the comparison of the control of this patient on tolbutamide and chlorpropamide after 10 weeks of study during each period. During both these 10-week periods dietary control was essentially the same. FIGURE 3 also shows that tolbutamide in doses of 4 gm. per day was quite effective in maintaining the patient's blood sugar control in the range of excellent (below 120 mg. per cent), while only a fair degree of control could be attained on 3.0 gm. of tolbutamide per day. No untoward reactions to tolbutamide were recorded during this time. Also shown is the fact that both 3.0 and 0.25 gm. of chlorpropamide per day quite effectively maintained the patient's fasting blood sugar levels in the excellent range.

Chlorpropamide was stopped after 4 weeks to determine the duration of effective levels of the drug. Three weeks after the drug was discontinued the fasting blood sugar levels rose from 100 to 150 mg. per cent and by the fifth week had risen to 163 mg. per cent. It was concluded that the duration of action of chlorpropamide was approximately 3 weeks, and that normal blood sugar levels could again be attained within 1 week after starting 0.25 gm. of chlorpropamide per day.

The first dose of 3.0 gm. of chlorpropamide reduced this patient's blood sugar level from 110 mg. per cent to 63 mg. per cent in 5 hours. On the third day of therapy at this dose level a rather marked hypoglycemic reac-

tion was produced during the early morning hours, with a blood sugar level of 57 mg. per cent. The diabetes was well controlled on maintenance levels of 0.25 gm. per day.

Mild side reactions were produced by 3.0 gm. of chlorpropamide per day. They included minimal confusion and weakness, moderate somnolence, and minimal anorexia that persisted for 2 to 3 days following reduction of chlorpropamide to 0.25 gm. per day, but rapidly disappeared thereafter. There

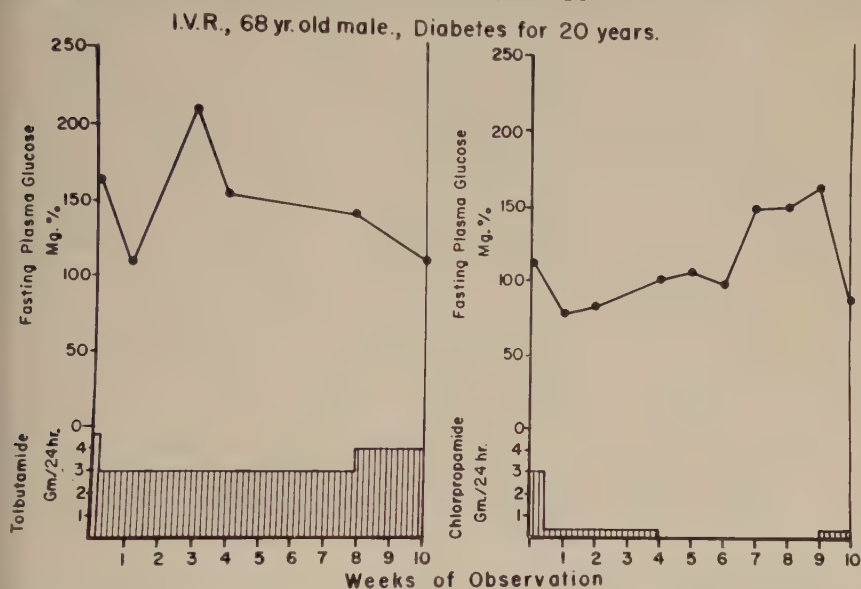


FIGURE 3. Comparison of the effect on fasting blood sugar levels of tolbutamide and chlorpropamide.

were no side reactions of any kind produced by the maintenance dose of 0.25 gm. daily.

The Effect of Chlorpropamide on Serum Cholesterol Levels

Serum cholesterol levels were determined on twenty-two patients receiving therapeutic doses of chlorpropamide. All patients were placed on calculated diabetic diets. TABLE 6 shows the serum cholesterol levels obtained at various times from this group.

It is of interest that the serum cholesterol level of 9 (41 per cent) of the 22 patients decreased by 50 mg. per cent or more while under treatment with chlorpropamide. The mean initial serum cholesterol level was 280 mg. per cent, and the mean level after 25 weeks of treatment was 239 mg. per cent—a mean decrease of 41 mg. per cent, which includes the mean decreases in 13 patients who did not show a decrease of 50 mg. per cent or more. There was a mean decrease of 79 mg. per cent in the 9 patients who showed a 50 mg. per cent or more decrease in serum cholesterol levels. The difference between these means was not statistically significant.

In almost all cases, the higher the serum cholesterol level prior to chlor-

propamide administration, the greater the fall in serum cholesterol level following chlorpropamide therapy. Evidence of this effect was further substantiated by the observation that in the 9 patients who showed a decrease in serum cholesterol of 50 mg. per cent or more, the mean serum cholesterol level prior to chlorpropamide therapy was 324 mg. per cent; in the 13 patients showing a decrease in serum cholesterol level of less than 50 mg. per cent, the mean initial level was 250 mg. per cent. There was no correlation

TABLE 6
SERUM CHOLESTEROL DETERMINATIONS MADE AT VARIOUS WEEKS AFTER STARTING CHLORPROPAMIDE THERAPY

Patient	Pre-treatment level, 1 wk.	Level, weeks after therapy																				Mean, following therapy
		2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	21		
1	222		231	205		253			231				248								234	
2	304	272		226	225																241	
3	352																				296	
4		290	255							192					218			242		221	225	
5	324							303		243						309					285	
6	238					200		181					206		194		179			177	189	
7	306				224								147								185	
8		294								256										244	250	
9	252		245									237		249							243	
10	266						283		257	276	275			276				275			273	
11	266		257		214			254				279			246		213			184	235	
12	267										239									235	237	
13	207		172	229		190						250			245						217	
14	270			224		228		179			271			291							238	
15	220					268		213				241		222						235	235	
16	248								254		247										250	
17	318			250			288	273		243											252	
18	194			193				157	129					170				209			162	
19	290					223	212		213			246							190		216	
20	272	264		222						212			210							261	234	
21	471			339				349	347												345	
22	288				181	269															225	
Mean	280																				239	

between the degree of blood sugar control and the fall in serum cholesterol level, nor was there a correlation between the fall in serum cholesterol, the duration of diabetes, or the amount of insulin previously required to control the diabetes.

Effect of Chlorpropamide on Thyroid Function

Twenty-three patients had repeated serum PBI levels while on chlorpropamide therapy. TABLE 7 shows the PBI determinations made at various weeks after starting chlorpropamide. The initial mean PBI level prior to chlorpropamide therapy was 5.8 μ g. per cent, and the mean level following chlorpropamide therapy was 5.4 μ g. per cent—a mean decrease of 0.4 μ g. per cent. The difference in these means was not statistically significant. At the end of 13 weeks of chlorpropamide therapy there was a slight decrease in serum PBI levels and, at the end of 25 weeks of therapy, there was an additional decrease.

Several cases warrant discussion because of marked decreases in their serum PBI levels while on chlorpropamide therapy. One patient showed a gradual decrease in serum PBI levels from 5.6 $\mu\text{g.}$ per cent to 3.6 $\mu\text{g.}$ per cent, with levels falling to less than 3 $\mu\text{g.}$ per cent on 2 occasions. A second patient showed a gradual decrease in serum PBI from an initial level of 6.0 $\mu\text{g.}$ per cent to 4.3 $\mu\text{g.}$ per cent after 19 weeks of therapy. Seven other patients showed a decrease in PBI levels of 1.0 $\mu\text{g.}$ per cent or more while on chlorpropamide treatment. As far as could be determined, no symptoms referable to these decreases in PBI levels were produced.

In an effort to determine whether chlorpropamide depressed the 24-hour uptake of RAI, 5 patients had control 24-hour uptakes and uptakes following administration of large doses (3 to 7 gm.) of chlorpropamide. RAI uptakes were also determined at hourly intervals and, when a plateau was reached, potassium thiocyanate (1.0 to 1.5 gm.) was administered. The mean pre-chlorpropamide 24-hour RAI uptake in the 5 patients was 18.1 per cent, and the mean 24-hour uptake following chlorpropamide administration was 11.4 per cent—a mean decrease of 6.7 per cent. The difference between means was not statistically significant. There was no significant discharge of RAI following the administration of potassium thiocyanate in any of the patients. It appears, then, that chlorpropamide, at least in large doses, may be capable of suppressing the 24-hour RAI uptake, but there is no evidence to suggest that chlorpropamide exerts any interfering action on the peroxidase enzyme system in a manner similar to propylthiouracil.

Effect of Chlorpropamide on Blood and Urine

All patients had complete peripheral blood evaluations and complete urinalyses at weekly intervals when first started on chlorpropamide therapy; the frequency of these determinations was decreased as therapy was prolonged.

At no time was there any evidence of anemia or granulocytopenia, although in a number of patients a slight increase in lymphocytes and monocytes was noted at approximately the same time the blood sugar fell toward normal. No pathological urine findings that could be attributed to chlorpropamide were noted in any of the patients.

Effect of Chlorpropamide on Liver Function

Three patients who had been maintained on relatively large doses of chlorpropamide for prolonged periods were evaluated with cephalin flocculation, thymol turbidity, and alkaline phosphatase tests. Four patients who had also been maintained for prolonged periods on large doses of chlorpropamide were evaluated with BSP tests. In none of these patients could any evidence of liver damage be found. Jaundice was not a finding in any of the fifty-two patients treated.

Untoward Effects

Although unpleasant to incapacitating side reactions occurred on all dose levels of chlorpropamide employed (0.25 gm. every other day to 3.0 gm. per

day), reactions were sufficiently severe in only 2 patients on 0.25 gm. per day to warrant discontinuance of medication.

TABLE 8 shows the percentage of side reactions in patients receiving various amounts of medication. There was an over-all incidence of side effects of some degree in 37 per cent of the patients, but only 15 per cent of the patients had sufficiently severe reactions to necessitate withdrawal of medication.

TABLE 9 reveals that weakness, hunger, dizziness, and somnolence were the most common complaints occurring on all dose levels used. The severity

TABLE 8
PERCENTAGE OF SIDE REACTIONS IN PATIENTS RECEIVING VARIOUS AMOUNTS
OF CHLORPROPAMIDE

Dose level* per day (gm.)	Number of patients	Number of patients having side reactions†	Percentage
3.00	3	2	67
2.00	13	12	92
1.50	2	1	50
1.00	13	7	54
0.50	39	24	62
0.25	32	15	47

* Several patients are included at more than one dose level.

† The side reactions in patients receiving 0.50 gm. or less of chlorpropamide were usually mild and relieved by food.

TABLE 9
ORDER OF FREQUENCY OF SIDE REACTIONS OBSERVED WITH CHLORPROPAMIDE
THERAPY

- | | |
|-----------------------|------------------|
| 1. Dizziness | 7. Skin eruption |
| 2. Weakness | 8. Confusion |
| 3. Hunger | 9. Headache |
| 4. Somnolence | 10. Anorexia |
| 5. Ataxia | 11. Vomiting |
| 6. Nausea | 12. Acidosis |
| 13. Hypoglycemic coma | |

of each of these 4 symptoms was related directly to the size of the dose used; the larger the dose, the more severe the complaint. These symptoms could not be correlated with hypoglycemia in all instances, but frequently—particularly in patients receiving 0.25 to 0.50 gm. of chlorpropamide per day—symptoms could be correlated with low blood sugar levels. Symptoms clinically resembling hypoglycemia could not be relieved by food ingestion when they were produced by 2.0 gm. per day or more of chlorpropamide, but could be relieved effectively by food ingestion when produced by doses of from 0.25 to 0.50 gm. per day. Dizziness, weakness, ataxia, somnolence, and confusion usually occurred together, and at no time were they the sole complaints. In all cases, these side reactions were most severe when they occurred on dose levels of from 1.0 to 3.0 gm. of drug per day; when produced

by doses of 0.5 to 0.25 gm. daily they were rarely incapacitating. These effects were usually produced within hours of drug ingestion when they accompanied doses of 1.0 to 3.0 gm. daily.

Severe somnolence was a complaint only in that group receiving 2.0 gm. or more of chlorpropamide daily. These patients, in the absence of pre-occupation, would immediately fall asleep, although they could be quite easily aroused and were not confused following arousal. Weakness was a prominent complaint in 2 patients receiving doses of chlorpropamide of 0.50 gm. per day or less and could not be relieved by the ingestion of food.

Ataxia, when it occurred, was usually present the day therapy was initiated, but was maximal the second to third day of treatment. Many patients frequently fell as a result of it. Relief was always rapid and complete after marked reduction of the dose of chlorpropamide. Only 1 of 32 patients receiving 0.25 gm. daily complained of ataxia; in this patient, the condition persisted for 1 week following withdrawal of the drug.

Nausea and vomiting occurred primarily in patients receiving 1.0 gm. or more daily of chlorpropamide and was rapidly and effectively relieved by decreasing the dose used. At no time was vomiting sufficiently severe to produce dehydration or electrolyte disturbance. When nausea and vomiting occurred in patients taking 0.25 to 0.50 gm. of chlorpropamide daily, it was usually correlated with poor control of diabetes.

Side reactions clinically resembling hypoglycemic reactions were commonly encountered in patients taking 0.25 to 0.50 gm. of chlorpropamide daily. Of these reactions, headache and hunger were quite common and, even though they frequently occurred together, they sometimes occurred independently. Characteristically they began late in the afternoon, just before the evening meal, and would be completely relieved by the ingestion of food. Infrequently, patients complained of being awakened by hunger during the early morning hours; food ingestion immediately prior to retiring prevented this effect. Somnolence, when it occurred in patients receiving 0.25 to 0.50 gm. of drug daily, frequently occurred after 6 to 8 weeks of therapy on chlorpropamide and appeared late in the afternoon, was associated with hunger and dizziness, and was readily relieved by food.

Skin rash and pruritis were very common reactions, occurring in 12 of 71 patients (17 per cent) receiving 0.25 to 0.50 gm. daily of chlorpropamide. One patient had severe erythema multiforme while being treated. A definite correlation existed between the duration of chlorpropamide therapy and the production of skin reactions; this explains why skin eruption was seen infrequently in patients taking 1.0 gm. or more of the drug daily, as only 2 patients were maintained on such doses for more than 2 weeks. These 2 patients did not experience side reactions of any type, which suggests the marked individual variation of susceptibility to this drug.

Five of 39 patients receiving 0.5 gm. of chlorpropamide daily complained of skin rash. The length of time patients were maintained on such a dose was considerably longer than the length of time patients were maintained on higher doses.

Seven of 32 patients taking 0.25 gm. daily of chlorpropamide had skin rash

and pruritis at one time during the course of therapy. Both of these effects were usually noticed within 3 weeks after initiation of therapy, and the incidence of these complaints increased as the duration of chlorpropamide therapy increased.

Skin rash characteristically was preceded or accompanied by pruritis, although this was not a constant finding. A small percentage of the patients complained of skin rash alone. When pruritis preceded the skin rash it invariably did so by 1 to 3 weeks and did not persist following disappearance of the rash.

The rash was usually a maculopapular eruption, erythematous, discrete, but never confluent. Characteristically, the face (particularly the malar eminences), the neck, the upper third of the chest (both anteriorly and posteriorly), and the proximal third of the arms were the areas included. When pruritis developed first, it was localized to these same areas. One patient developed a localized pretibial eruption. The skin rash was usually transient and of only 2 to 14 days' duration, disappearing completely after this time. Two patients were exceptions to this; one, after being treated with 0.5 gm. of chlorpropamide daily for 3 weeks, developing an itching, red rash. Chlorpropamide therapy was immediately withdrawn, but the rash continued to increase in severity, and 4 weeks later a severe exfoliative dermatitis developed with profuse vaginal and urethral discharge, labial ulcers, and mouth ulcers. The patient became extremely ill although, following hospitalization for 2 weeks and extensive treatment, she did well and was discharged without sequelae. A second patient was treated with 0.5 gm. of chlorpropamide daily for 1 week. The drug was then withdrawn because of the development of nausea, weakness, ataxia, and dizziness. One week after discontinuance of the drug the patient developed an itching, urticarial type of eruption that responded only minimally to extensive antihistaminic treatment. The rash progressed for 5 weeks and disappeared by the end of 7 weeks; recovery again was complete.

Acidosis occurred in only two patients, neither of whom had a blood sugar response to chlorpropamide. In both patients it developed two days after treatment was started. One patient was a juvenile diabetic with a past history of acidosis, the other an adult diabetic without such previous history.

Hypoglycemic coma occurred in 1 patient, a 60-year-old Negro male who had previously been poorly controlled on 130 U. of NPH U-80 insulin daily. At the time of development of hypoglycemic coma he was receiving 1.0 gm. of chlorpropamide per day. Symptoms developed during the early morning hours after he had failed to eat the evening meal the preceding night. On admission to the emergency room his blood sugar was 30 mg. per cent. He responded immediately to intravenous glucose and has been well controlled since on 0.5 gm. of chlorpropamide per day without further complaint.

Death occurred in one patient being treated with chlorpropamide, but as this patient had her terminal treatment in an outlying hospital, the details surrounding her death are unknown. An autopsy was not obtained but she had numerous, severe complications such as congestive heart failure, and chlorpropamide was thought not to be related to her death.

Summary and Conclusions

Studies are reported on 52 randomly selected adult patients with diabetes mellitus who were treated from 1 to 25 weeks with 0.25 to 3.0 gm. daily of chlorpropamide. Thirty patients were maintained on this drug for from 17 to 25 weeks.

Fair to excellent blood sugar control was obtained in 28 (54 per cent) of the patients treated. The best response was obtained in those patients with a requirement of less than 21 U. of insulin daily for control of the diabetes.

One patient was treated with both chlorpropamide and tolbutamide, and it was found that chlorpropamide was approximately 4 to 8 times as potent as tolbutamide in reducing this patient's blood sugar levels. No untoward effects were noted from either of these drugs in this patient.

A mean decrease in serum cholesterol level of 41 mg. per cent was observed in twenty-two patients following treatment with chlorpropamide. This decrease was not statistically significant. A tendency for serum cholesterol to be lowered by chlorpropamide when initial serum cholesterol levels were high was suggested. The duration of diabetes, previous control of diabetes, and the amount of insulin previously required for control played little or no part in determining the response of cholesterol to chlorpropamide.

Twenty-three patients were studied to observe the effects of chlorpropamide on thyroid function. A mean decrease in serum PBI levels of 0.4 μ g. per cent was observed following 25 weeks of treatment with chlorpropamide; this was not statistically significant. Five patients were observed to have a mean decrease of 6.7 per cent in 24-hour RAI uptakes following chlorpropamide treatment but, again, this was not statistically significant.

Untoward effects were common with chlorpropamide treatment and, in general, tended to be most severe and most frequent on dose levels of 1.0 gm. or more, daily. While these reactions were due to hypoglycemia in some instances and could be relieved by food ingestion, in many instances they appeared to be the result of a direct effect of chlorpropamide. Side reactions were also commonly observed on maintenance-dose levels of 0.25 to 0.50 gm. daily, but these were usually relieved by the ingestion of food and were thus transient and of little consequence.

Renal and hematological damage was not found with chlorpropamide therapy, nor was liver function impairment observed.

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CLINICAL EXPERIENCE WITH CHLORPROPAMIDE IN THE MANAGEMENT OF DIABETES MELLITUS*

A. Gorman Hills and William A. Abelove†

Section of Endocrinology and Metabolism, Department of Medicine, University of Miami School of Medicine, Coral Gables, Fla., and the Diabetic-Endocrine-Metabolic Clinics, Jackson Memorial Hospital, Miami, Fla.

Introduction

This report is based upon our observations of the compound 1-propyl-3-(*p*-chlorobenzenesulfonyl)urea (chlorpropamide) orally administered as an agent for the control of glycosuria and hyperglycemia as tested in 49 patients with diabetes mellitus for periods of 2 to 5 months. The drug has been found to be effective for this purpose in 76 per cent of this group, most of whom were patients whose diabetes began in their fifth decade or later. The data have been analyzed in terms of an arbitrary system for grading the degree of control achieved with the aid of the drug, and an appraisal on this basis of clinical factors influencing the effectiveness of the drug has been made. The concentration of chlorpropamide in blood serum attained at various dosage levels has been determined also, and the relation of these two factors to the effectiveness and toxicity of the drug has been evaluated.

Clinical and Experimental Data

Of the 49 patients included in this study, 35 were females and 14 males. All but 6 of the patients previously had required insulin, and 14 of these had been treated with tolbutamide and had been adequately controlled by that compound. The remaining 6 patients were new diabetics, 2 having been controlled on tolbutamide prior to chlorpropamide administration; in all 6, the blood sugar levels had been constantly higher than 195 mg./100 ml. before drug treatment, and good control was obtained by treatment in all 6.

Ten of the patients were studied initially for several weeks as inpatients in the Special Study Unit of Jackson Memorial Hospital. During the study they were kept on constant diet, and the excretion of glucose and ketones in the urine was determined quantitatively on partitioned 24-hour urine collections. Fasting blood sugar was also determined daily. After a suitable control period of observation, chlorpropamide therapy was begun, usually as 0.5 gm. twice daily for two days, with subsequent maintenance on a single daily dose. Insulin, when it was being given during the control period, was withdrawn within 2 to 4 days of initiation of chlorpropamide therapy, with initial daily decrements of about 50 per cent.

The remainder of the subjects were followed as outpatients, and their diets

* The study reported in this article was supported in part by a grant from Chas. Pfizer & Co., Inc., Brooklyn, N. Y.; by Research Grant H-2533 from the National Heart Institute, Public Health Service, Bethesda, Md.; and by a Graduate Training Grant from the National Institute of Arthritis and Metabolic Diseases, Public Health Service, Bethesda, Md.

† Trainee (1957-1958) of the National Institute of Arthritis and Metabolic Diseases, Public Health Service, Bethesda, Md.

were controlled only insofar as they adhered to their diet prescription. They were seen at weekly intervals until their drug therapy and the response thereto were stabilized, and subsequently at intervals of two to three weeks.

If good control was achieved with 500 mg. of chlorpropamide daily, the maintenance dose was lowered; if satisfactory control was not achieved with 500 mg. daily, the dose was increased.

Determinations of chlorpropamide concentration in blood sera were made in both outpatients and inpatients with the aid of the method of Toolan and Wagner.¹

TABLE 1 summarizes the status of the 49 patients with respect to previous treatment.

TABLE 1
NUMBER OF PATIENTS ACCORDING TO PREVIOUS THERAPY

Insulin.....	29
Insulin, then tolbutamide.....	14
Tolbutamide.....	2
New diabetics.....	4
Total.....	49

Criteria of Control

Criteria for satisfactory control were arbitrarily established as follows: good, fasting true blood sugar consistently below 120 mg./100 ml., with no glycosuria; fair, fasting blood sugar between 120 and 170 mg./100 ml., with glycosuria less than 0.5 per cent.

Analysis of the Data

Age at onset of diabetes. The effectiveness of chlorpropamide in controlling hyperglycemia is most striking in those patients in whom diabetes mellitus first made its appearance after the age of 40. Of 43 patients in this category who were given the drug, 36, or 84 per cent, were adequately controlled without insulin. The excellent response often observed in these patients is exemplified in FIGURES 1 and 2.

In 5 patients whose diabetes began between the ages of 20 and 39, however, therapy was successful in only 1. The total lack of response encountered in patients of this group is illustrated in FIGURE 3. Chlorpropamide was also ineffective in the 1 child among our patients who received the drug, a 14-year-old boy with diabetes of 5 years' duration, who required 52 U. of lente insulin daily. TABLE 2 gives the age-at-onset data for the 49 patients.

Duration of diabetes. As shown in TABLE 3, no significant influence of the duration of disease upon responsiveness to chlorpropamide could be detected. The somewhat lower incidence (50 per cent) of satisfactory control in patients with a history of diabetes of 15 years or more probably represents random variation in the sample, for the number of patients is small and, if attention is confined to 4 of these patients who had had diabetes more than 20 years, the drug is shown effective for 75 per cent.

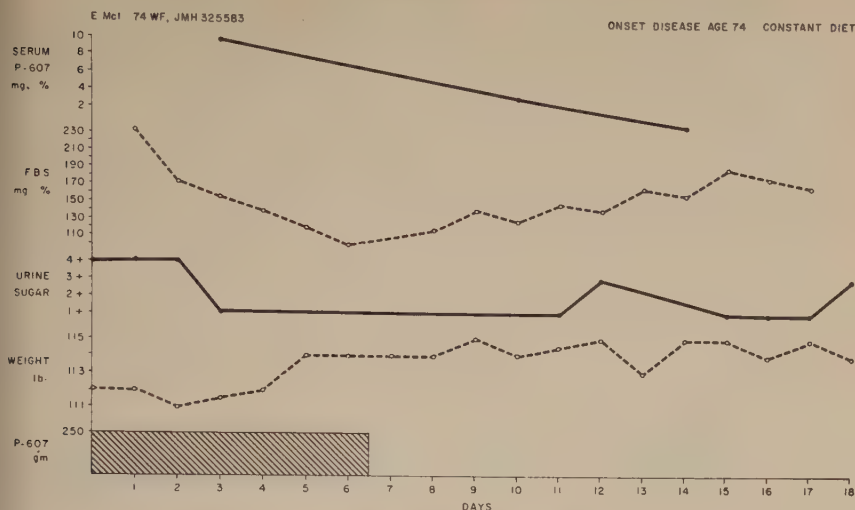


FIGURE 1. Response of a previously uncontrolled diabetic to chlorpropamide. After a control period on a constant diet, chlorpropamide therapy was begun without other change of regimen. It should be noted that optimum control, in terms of the blood sugar level, occurred after six days of therapy, and that complete relapse had not occurred twelve days after drug therapy was stopped. The serum chlorpropamide concentration was still appreciable one week after cessation of therapy. "FBS" refers, in all the charts, to the fasting blood sugar, and P 607 to chlorpropamide.

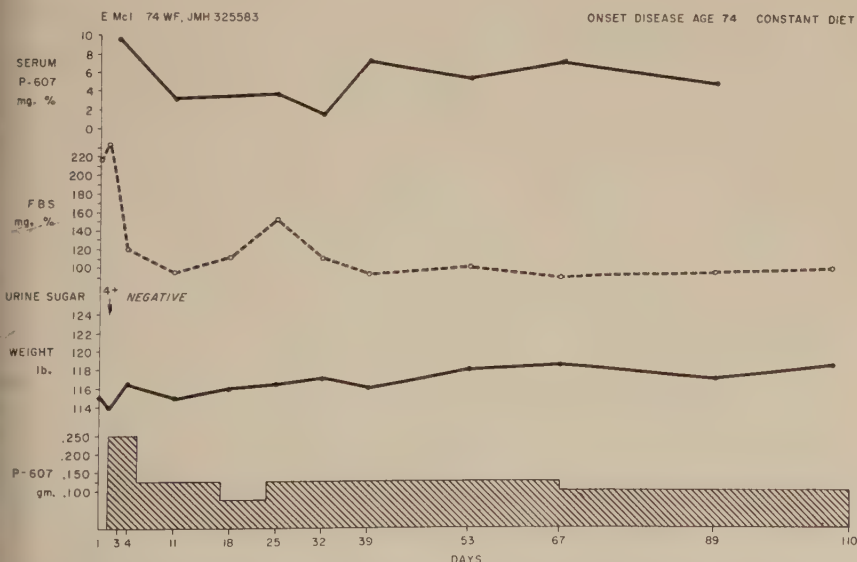


FIGURE 2. Effects of resumption of chlorpropamide therapy in the patient of FIGURE 1. Control for a 3½-month period has been excellent at a low dose level in this patient with newly diagnosed diabetes mellitus.

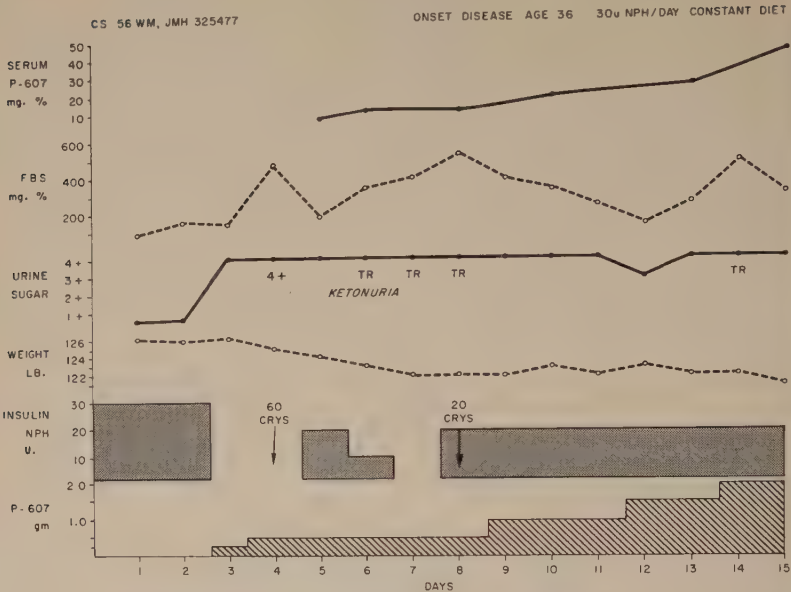


FIGURE 3. Primary failure with chlorpropamide. On constant diet in the special study unit, this patient was excellently controlled on 30 U. of NPH insulin daily. Hyperglycemia, severe glycosuria, weight loss, and ketosis were not prevented by doses of chlorpropamide (to 2.0 gm. daily) supplemented by 20 U. daily of NPH insulin.

TABLE 2
RELATION OF AGE AT ONSET OF DIABETES AND RESPONSE TO THERAPY

	Age at onset, years				Total patients	Percentage
	0-19	20-39	40-59	60-80		
No. of patients.....	1	5	22	21	49	—
Control:						
Good.....	0	1	10	9	20	41
Fair.....	0	0	8	9	17	35
Unsatisfactory.....	1	4	4	3	12	24
Percentage satisfactory.....	0	20	82	86	—	—

Previous insulin requirement. There was no doubt that the effectiveness of chlorpropamide was related to the previous insulin requirement (TABLE 4). Good control was obtained in all patients who had not previously required insulin. Control was satisfactory (not always good) in 85 per cent of patients who had required less than 20 U. of insulin daily; where 20 to 40 U. had been required, 71 per cent were satisfactorily controlled, with relatively few well controlled. Our experience with those requiring more than 40 U. of insulin daily was limited to two patients. One was the juvenile mentioned above.

the other a 68-year-old white female with an onset age of fifty, requiring 80 U. of NPH insulin daily. On a daily dose of 500 mg. chlorpropamide per day, her insulin requirement was reducible to 50 U. of NPH daily, but no lower.

Comparison of tolbutamide and chlorpropamide. In 15 patients previously maintained on tolbutamide in doses of 0.5 to 2.5 gm., chlorpropamide was substituted (TABLE 5). Thirteen of these had been on insulin prior to the tolbutamide; 2 were new diabetics. The effective dose of chlorpropamide

TABLE 3
RELATION OF DURATION OF DIABETES AND RESPONSE TO THERAPY

	Duration of diabetes, years					Total patients	Percentage
	1	1-5	6-9	10-15	15		
No. of patients.....	7	18	6	12	6	49	—
Control:							
Good.....	5	8	2	2	3	20	41 } 76
Fair.....	0	6	4	7	0	17	
Unsatisfactory.....	2	4	0	3	3	12	24
Percentage satisfactory.....	71	78	100	75	50	—	—

TABLE 4
RELATION OF INSULIN REQUIREMENT AND RESPONSE TO CHLORPROPAMIDE THERAPY

	Previous insulin dose, units per day				Total patients	Percentage
	0	Less than 20	20-40	Greater than 40		
No. of patients.....	6	13	28	2	49	—
Control:						
Good.....	6	7	7	0	20	41 } 76
Fair.....	0	4	13	0	17	
Unsatisfactory.....	0	2	8	2	12	24
Percentage satisfactory.....	100	85	71	0	—	—

averaged approximately one-fifth that of tolbutamide; a typical experiment is shown in FIGURE 4. One patient (FIGURE 5) showing progressive resistance to tolbutamide was shifted to chlorpropamide; hyperglycemia and ketosis continued, and insulin was required for control.

Sex and race. There was no correlation between the sex or race and response to therapy (TABLE 6).

Dosage of chlorpropamide. The majority of our patients were maintained satisfactorily on doses of 500 mg. per day or less, generally given as a single

TABLE 5
COMPARISON OF TOLBUTAMIDE AND CHLORPROPAMIDE DOSAGE

	Previous maintenance dose (tolbutamide), grams per day					Total patients
	0.5	1.0	1.5	2.0	2.5	
No. of patients converted.....	1	3	6	4	1	15
Present maintenance dose (chlorpropamide), grams per day						
0.100.....	1	—	2	—	—	
0.200.....	—	2	3	—	—	
0.250.....	—	—	1	1	—	
0.400.....	—	1	—	1	—	
0.500.....	—	—	—	—	1	
0.625.....	—	—	—	2	—	

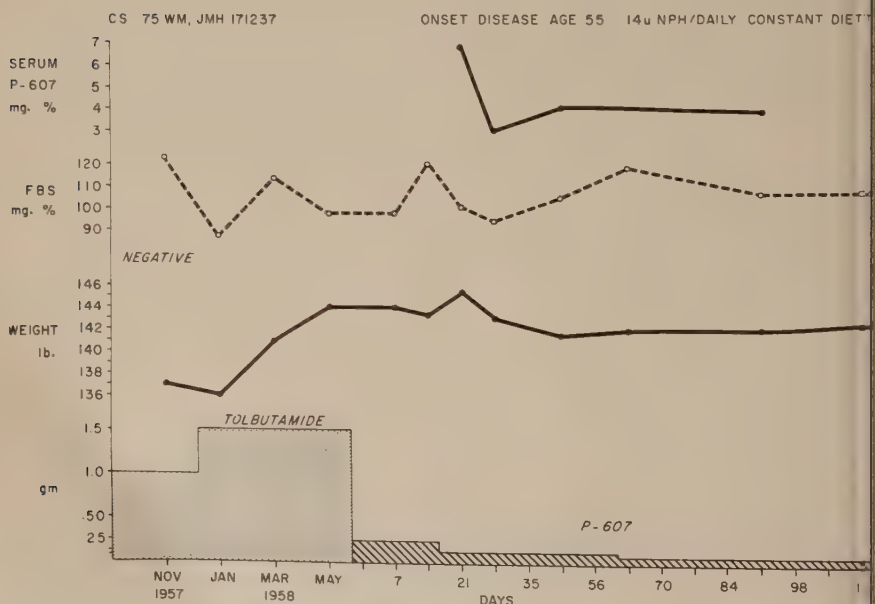


FIGURE 4. Replacement of tolbutamide by chlorpropamide. The observations illustrated were made while the patient was on a constant diet in the special study unit. Chlorpropamide, in a dose of 125 to 250 mg. daily, maintained the satisfactory control previously achieved in this patient by 1 to 1.5 gm. tolbutamide daily.

dose each morning. It was observed that patients taking small doses (that is, 100 mg. per day) could be controlled just as readily when the dose was given as a 200-mg. tablet every other day (TABLE 7). In view of the relatively slow elimination of the drug, it is probable that the same thing may be said of higher doses, but we have no observations to support this assertion.

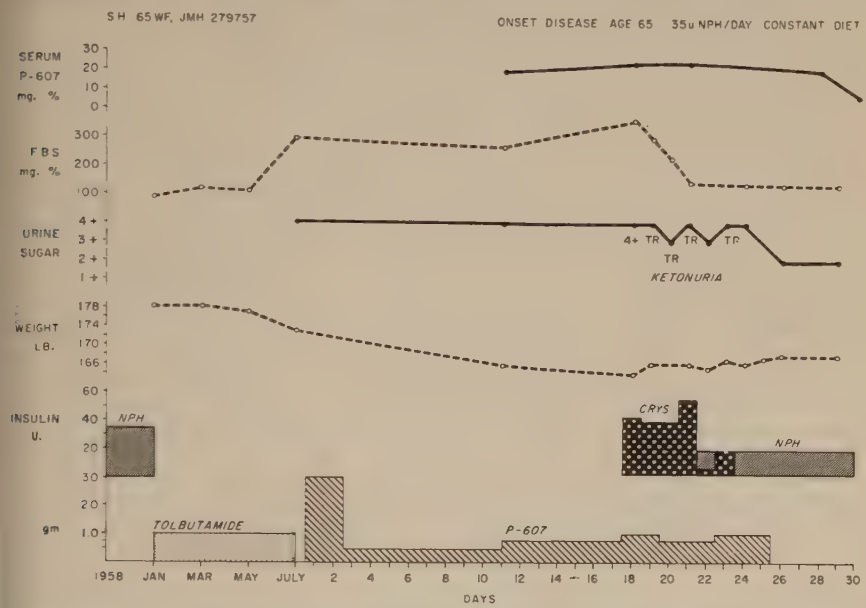


FIGURE 5. Failure of chlorpropamide in tolbutamide resistance. Neither tolbutamide (1 gm. daily) nor chlorpropamide (750 mg. daily) produced successful control of diabetes in this patient.

TABLE 6
RELATION OF SEX AND RACE TO SUCCESS OF THERAPY

	Male	Female	White	Negro
No. of patients.....	14	35	37	12
No. satisfactorily controlled.....	11	26	29	8
Percentage satisfactorily controlled..	79	74	78	67

Diabetics not responding to 500 mg. per day usually failed to respond to doses of as much as 2 gm. per day. At these higher dose levels toxic symptoms were not uncommon.

Concentration of chlorpropamide in blood serum. When chlorpropamide is administered in a constant daily dose of 1 gm. or less, the serum concentration of the drug rises steadily for about five days, attaining a reasonably stable plateau by the end of that time. The decline in serum concentration when

the drug is stopped is still slower, and this is reflected in a rather delayed escape from diabetic control in patients who cease taking the drug (FIGURE 1).

FIGURE 6*a* represents the range of serum chlorpropamide concentrations found in a number of patients stably maintained at various dose levels of the drug. To these data has been fitted freehand the straight-line curve shown in FIGURE 6*b*. The assumption, which may not be entirely accurate, has been made that the relation between drug dose and serum concentration is linear. At any rate, FIGURE 6*a* gives a rough indication of the drug concentration to be expected at any dose level.

TABLE 7
RELATION OF MINIMAL DOSE TO ADEQUATE CONTROL*

	Chlorpropamide dose (grams per day)			Total
	0.1-0.2	0.25-0.50	0.60	
No. of patients.....	20	13	16	49
Control:				
Good.....	14	6	—	20
Fair.....	6	6	5	17
Unsatisfactory.....	—	1	11	12

* Satisfactory control was achieved in 33 of the 49 patients (67 per cent) by a daily dose of 500 mg. of chlorpropamide or less and, where 500 mg. sufficed, more often than not 200 mg. or less gave equally satisfactory control. Five patients (10 per cent) in whom control had been unsatisfactory at a daily dose of 500 mg. were brought under control by higher doses, but good control was not achieved in any of these patients with doses as high as 2 gm. daily.

TABLE 8 shows that satisfactory control, if it can be attained at all, will generally be achieved at a plasma concentration of chlorpropamide of 15 mg./100 ml. or less. According to FIGURE 6*b*, such a concentration would be expected in a person weighing 65 kg. and taking 520 mg. of the drug daily, although there is a good deal of variability in and between individuals.

Thirty-six of the patients represented in TABLE 8 have had repeated determinations of serum chlorpropamide concentration at a stable dose level of the drug; in 3 of these, a second series of observations was made at higher dose levels and with higher serum concentrations.

Of the 36 patients, 22 (61 per cent) were satisfactorily controlled at maximum serum concentrations of 15 mg. or less per 100 ml. Three patients not satisfactorily controlled at the lower serum concentrations (15 mg./100 ml. or less) were subsequently given larger doses of the drug. One remained uncontrolled with serum concentrations as high as 36 mg./100 ml.; fair control was achieved in the other 2 at maximum levels of 20 and 25 mg./100 ml. respectively.

Toxicity. Toxic manifestations occurred in 15 of our 49 cases (30 per cent);

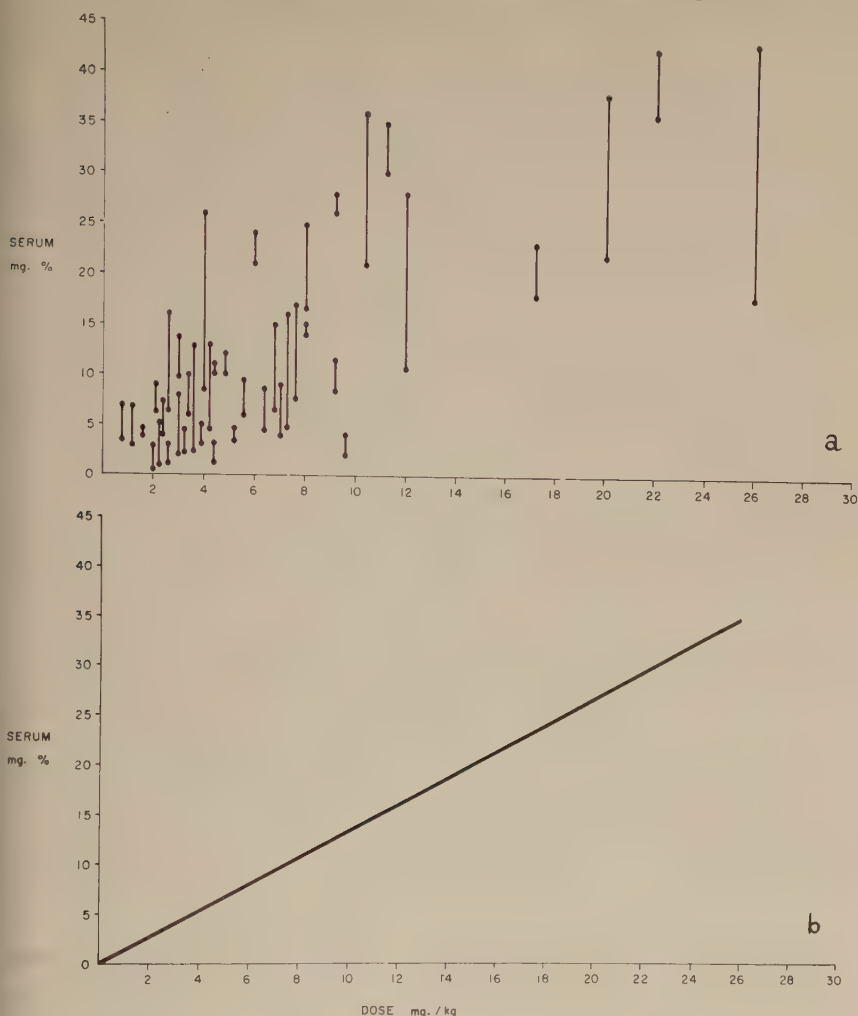


FIGURE 6. Relation of serum chlorpropamide levels to oral dosage. (a) Each bar shows the range of serum chlorpropamide concentrations attained 4 days or more after any given constant daily dose of the drug had been instituted. The data are based on 145 determinations in 39 patients, each range shown representing, on the average, 3.7 determinations carried out for weeks or months at a stable dosage level. (b) A free-hand curve drawn to fit the data of FIGURE 6a.

Most of the effects were transient and consisted of headache, dizziness, drowsiness, itching, and possible hypoglycemic symptoms (TABLE 9). There were, however, only 2 instances (4 per cent) where the symptoms persisted and necessitated withdrawal of the drug. In 1 patient (G. E.), who had been taking 500 mg. per day with marginal fair control, the dose was increased to 750 mg. per day, with ensuing nausea, vomiting, hiccoughs, and an erythem-

atous maculopapular eruption. Therapy was discontinued, and the eruption cleared within 10 days. In another patient (L. W.), poorly controlled on 500 mg. per day for 7 days, the dose was increased to 1 gm. per day. Within 3 days, a mild exfoliative dermatitis had appeared. Insulin was substituted for chlorpropamide, and the condition cleared within 2 weeks.

TABLE 8
RELATION OF BLOOD LEVEL TO CONTROL*

	Serum chlorpropamide (mg. per cent)							
	1-5	6-10	11-15	16-20	21-25	26-30	31-40	41-50
Control:								
Good.....	5	9	—	1	—	1	—	—
Fair.....	2	1	5	2	1	2	—	—
Unsatisfactory.....	1	1	1	—	2	—	3	2

* Based on the highest serum concentrations attained at any given dose level.

TABLE 9
TOXIC REACTIONS TO CHLORPROPAMIDE

Type	No. of patients	Dose (gm./day)	Serum chlorpropamide (mg. per cent)	Transient	Persistent	Therapy discontinued
Dermatological						
Itching (no rash)	2	0.25	4.5	2	0	0
Rash.....	1*	1.0			1*	1*
Exfoliative dermatitis	1	0.75	9.1		1	1
Gastrointestinal						
Nausea, vomiting, and hic- coughs	1*	1.0				
Central Nervous System						
Headache.....	2	0.75	14-20	2	0	0
Dizziness, drowsiness.....	5	1.0	30-35			
Hypoglycemic symptoms	4	0.2-0.750	5.2-20.0	5	0	0
		0.500	26.0	4		0
		0.200				
		0.200	9.8			
Totals.....	15	0.200	13.6	13	2	2

* Same patient.

Symptomatic hypoglycemia, promptly relieved by sugar ingestion, was reported by four outpatients taking the drug, but we have not had the opportunity to document the occurrence of hypoglycemia by blood sugar determinations.

Most of the toxicity occurred when more than 500 mg. per day of the drug was being administered, and with serum concentrations of the drug of 10 mg./100 ml. or more, especially with levels higher than 15 mg./100 ml.

In all patients hemoglobin, erythrocyte count, leukocyte count with differential count, platelet count, and urinalysis were done; blood urea nitrogen, serum bilirubin, alkaline phosphatase, and thymol turbidity were determined prior to institution of drug therapy. No evidence has yet been obtained to suggest that the drug has caused any damage to the kidneys, liver, or bone marrow in any of our patients, and all mentioned determinations are being systematically rechecked several months after therapy is begun.

Comment

Our observations afford convincing evidence that chlorpropamide is an effective agent for the control of most cases of diabetes mellitus originating after the age of 40 years and requiring 40 U. or less of insulin daily under ordinary circumstances. By "control" is meant amelioration of glycosuria and reduction of blood sugar concentrations to the specifications set forth above; the drug is ineffective in the presence of acute complications of diabetes. Nothing in these data, of course, sheds any light on the physiological mechanism by which the observed effects are produced in the diabetic patient, nor necessarily implies correction by the drug of the derangement or derangements of intermediary metabolism which originally underlay the hyperglycemia.

In particular, our observations do not disclose whether chlorpropamide exerts its effects by a mechanism similar to that of other oral hypoglycemic agents now under close scrutiny by physicians and physiologists. There is, nevertheless, a striking similarity between chlorpropamide, as we have observed its effects, and the heretofore most widely studied oral hypoglycemic agent, tolbutamide. The latter drug, too, has found its principal usefulness in patients in whom diabetes has developed after the age of 40 and who require 40 U. or less of insulin daily for control. Indeed, the figures for percentile effectiveness of chlorpropamide under various circumstances (TABLES 2, 3, and 4) are very similar to corresponding observations of our own and of others^{2, 3} for tolbutamide.

When comparison is made in terms of the minimal maintenance dose required for control of diabetes, chlorpropamide is about five times more potent than tolbutamide on a weight basis. Much if not all of the difference is to be ascribed to the relatively slow rate of elimination of chlorpropamide, which is reflected, when administration of the drug is stopped, in a slow rate of decline of the serum chlorpropamide concentration, together with a delay in the reappearance of signs of metabolic decompensation. From the therapeutic standpoint, this characteristic of the drug confers upon it both the advantages and disadvantages of relative stability. Medication can be taken on a less precise schedule than in the case of a rapidly excreted material; in fact, it appears that stable control can be achieved regularly by giving the entire dose requirement for forty-eight hours at two-day intervals. On the other hand, a relatively longer period is required, after toxicity has appeared, to rid the body of the drug.

Toxic manifestations, especially those involving the central nervous system, occur more frequently when serum concentrations of the drug in excess of

15 mg./100 ml. are reached. The maximum recommended dose is generally 8 mg./kg. per day (500 mg. per day for the average-size patient). Further increases seldom give more effective control, and they will often raise the serum chlorpropamide level to a range higher than 15 mg., where a considerable increase of toxic reactions is to be expected. It is evident, however, that the dose of the drug per kilogram of body weight can influence effectiveness and toxicity only insofar as it affects the concentration of the drug in body fluids. Since the data show that there is considerable variation between individuals in the serum drug concentration attained at any given dose, it would appear that the determination of serum chlorpropamide concentration will often be helpful in establishing the optimal dose more precisely and, especially, in justifying increases to more than 8 mg./kg. per day in patients who, on this amount of drug, may remain uncontrolled and maintain low serum drug concentrations.

The incidence of toxic reactions of some kind in our series has been high, but our figure of 30 per cent does not, of course, represent the incidence of toxicity to be expected in patients who will be treated after the principles of proper dosage have become well established. In the course of preliminary, experimental clinical studies of a new drug, neither the minimum effective dose nor the incidence of toxicity at various dose levels has yet become well defined; and the majority of the toxic manifestations reported here have occurred at a higher dosage than would now ordinarily appear to be indicated.

Summary

Forty-nine patients with diabetes mellitus, ranging from fourteen to eighty years of age have been treated with chlorpropamide for as long as five months.

Satisfactory control of the diabetes was achieved in 76 per cent of these cases, the majority of patients being those whose disease began after 40 and who required less than 40 U. of insulin daily. The incidence of successful control was not significantly influenced by the sex or race of the patients nor by the duration of the diabetic state.

Chlorpropamide is effective in controlling diabetes, in general, under the same circumstances as tolbutamide; however, chlorpropamide is about five times more potent on a weight basis. This enhanced potency appears to reflect a relatively slow rate of disposal of chlorpropamide present in body fluids; it is also reflected in a delay of several days, after maintenance therapy is stopped, of escape from control.

Few patients not controlled at serum chlorpropamide concentrations of 15 mg./100 ml. will be well controlled at higher serum drug levels. A daily chlorpropamide dose of 8 mg./kg. (about 500 mg. per day for an average-size person) will generally result in such a serum concentration of the drug, but there is considerable variation, especially between individuals. For this reason, serum chlorpropamide determination should prove a helpful guide to therapy.

Toxic manifestations of some kind, principally mild and all reversible, occurred in 30 per cent of our cases. The incidence of toxic reactions in this

series is higher than, and not representative of, that to be expected with therapeutic use of the drug, which in our opinion should ordinarily consist of daily doses not in excess of 8 mg./kg. of body weight.

Acknowledgment

We are indebted to Domenic G. Iezzoni of Chas. Pfizer & Co., Inc., Brooklyn, N. Y., for supplies of chlorpropamide.

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CLINICAL AND EXPERIMENTAL STUDIES WITH CHLORPROPAMIDE IN DIABETES MELLITUS, IN NORMAL INDIVIDUALS, AND IN NONDIABETICS WITH HEPATIC DISEASE

Bernard Knick

Medical Clinic of the University of Mainz, Mainz, Germany

The hypoglycemic action of the sulfonylureas presupposes reserves of endogenous insulin. These substances probably also act through an extra-pancreatic mechanism influencing intrahepatic metabolism, a process that has been confirmed clinically and experimentally by a selective accumulation of liver glycogen. Because the sulfonylureas produce no increase in sugar utilization by skeletal muscle tissue, their action differs significantly from the action that is typical of exogenous insulin. The liver, therefore, is one of the sites affected by sulfonylureas.

It has been clinically and experimentally shown that the sulfonylureas influence intrahepatic metabolism. The mode of action of chlorpropamide, as proved in our studies, does not differ significantly from that of carbutamide or tolbutamide. In test animals the antidiabetic drugs, including chlorpropamide, produce a similar increased accumulation of glycogen in the liver. In rats with liver damage experimentally induced by thioacetamide (35 mg. per day by oral tube), chlorpropamide, like the other antidiabetic substances, exhibits a liver-protecting action. In nondiabetics with chronic hepatic disease (hepatitis and cirrhosis), we observed a similar effect and, also, an objective improvement in hepatic function; the latter was confirmed by laboratory and liver function tests, especially Bromsulphalein retention. Adverse effects on hepatic function were not demonstrated in these patients, nor in 35 diabetics treated with chlorpropamide orally administered for several months. In rats receiving 50 mg. or 100 mg./kg. of chlorpropamide for 6 weeks, we observed an increase in liver glycogen, but no histopathological alterations.

Because of the recent interest in the mechanism of action of the antidiabetic sulfonylurea derivatives, the effect of chlorpropamide on the liver transaminase system was investigated, since an inhibition of transaminase reactions can lead to a decrease in the rate of neoglucogenesis. Serum transaminase values were determined in normal individuals, hepatic patients, and diabetics during therapy. Glutamate-oxalacetic and glutamate-pyruvate transaminase levels, as well as aldolase and lactic acid-dehydrogenase activity, were investigated. It was observed that chlorpropamide inhibited serum transaminase levels as do other compounds, as shown by other investigators. It is suggested that by inhibiting transamination such derivatives decrease the rate of neoglucogenesis. This hypothesis would also explain the hepatotropic effects.

In our work at the Medical Clinic we have been particularly interested in determining minimal maintenance dosages. We have under study 38

patients, 25 of whom received previous treatment and 10 of whom were diagnosed during the course of the study and received chlorpropamide as the initial therapeutic agent.

Six of the patients were less than 25 years old, and it was in this group that we observed our 2 failures. The other 4 patients are interesting, since we did not observe an adequate response during the initial 2 weeks of treatment and, therefore, gave insulin in addition to chlorpropamide. We continued this combined therapy for 14 to 21 days, after which we found, surprisingly, that the insulin dosage could be reduced and finally discontinued.

Our remaining 29 patients, most of whom were 50 years of age or older and generally obese, responded well to chlorpropamide. It is our practice to begin treatment with 1 gm. per day for 3 or 4 days and then to reduce dosage to the lowest possible therapeutic level. We have found that most patients can be maintained in satisfactory balance with a dosage of 250 mg. daily. However, we have had success with as little as 100 mg. daily and, in an occasional patient, with 100 mg. every other day.

We have not observed any side effects from dosages of 1 gm. daily or less. On higher dosage, patients may experience nausea, discomfort in the epigastrium, and a feeling of abdominal fullness. There was no indication of clinical toxic manifestations on the blood, skin, or nervous systems.

We were impressed with the relatively low minimal dosages of chlorpropamide that were fully effective in maintaining satisfactory sugar metabolism in patients with diabetes. Tolerance of dosages of 1 gm. a day or less is excellent; probably, more than that amount is not necessary, since it may well represent the maximum therapeutic quantity. The average maintenance dosage is 250 mg. daily and, not infrequently, even smaller doses are adequate.

EXPERIENCES WITH CHLORPROPAMIDE, ESPECIALLY IN THE BRITTLE DIABETIC

Joseph J. Lowenthal, Carl Ross, George Tully

Diabetic Clinic, Duval Medical Center, Jacksonville, Fla.

It has been shown that chlorpropamide is a sulfonyleurea with antidiabetic action. Early in our clinical trial of this drug, we attempted to evaluate its control of the brittle or labile diabetic. We also attempted to determine whether the administration of very large doses would result in a more rapid control of the diabetes, and whether very large maintenance doses would control the brittle diabetic patient when average dosages failed to do so. Thus far, 34 patients with diabetes mellitus have been given chlorpropamide under our supervision. All of these patients were adhering to a restrictive diet of the American Diabetic Association type; 11 were taking, in conjunction with the diet, more than 34 U. of insulin daily in order to achieve control of their diabetes.

Of the 34 diabetic patients receiving chlorpropamide therapy, the drug was discontinued in 5 because of ineffectiveness of control, and in 5 others because of untoward effects. One patient was lost to our control, and 1 patient died.

Initial Dosage

Twenty-one patients were given an initial dosage of more than 1 gm. of chlorpropamide daily. Of these, 12 achieved adequate control. Two patients received 4 gm. as an initial dosage; neither achieved adequate control. Ten received 3 gm. as an initial dosage; of these, 6 were inadequately controlled. Five patients received initial dosages of 2 gm.; 3 of these were inadequately controlled (TABLE 1).

TABLE 1
CONTROL ACHIEVED WITH MORE THAN 2.0 GRAMS OF CHLORPROPAMIDE AS INITIAL DOSE

Initial dose (gm.)	Number of patients	Control	
		Adequate	Inadequate
2.0	5	3	2
3.0	10	4	6
4.0	2	0	2

Maintenance

Seven of our patients received maintenance dosages of more than 1 gm. daily; of these, 3 had adequate control. Of 3 patients receiving more than 2 gm. and as much as 4 gm. daily for maintenance, none was controlled.

One of these patients received 4 gm. daily for 4 days and achieved a blood level of chlorpropamide of more than 50 mg. per cent. Although she had no toxic effects or symptoms from this drug, it was discontinued because of lack of control (TABLE 2).

TABLE 2
MAINTENANCE DOSE (CHLORPROPAMIDE ONLY) AND RESULTS ACHIEVED

Maintenance dose (gm.)	Number of cases	Control	
		Adequate	Inadequate
0.25	1	1	
0.25-0.5	14	12	2
0.6-1.0	12	6	6
1	4	3	1
2-3	0	0	0
3	0	0	0
4	3	0	3

Control

Of 11 patients requiring a restrictive diet and more than 34 U. of insulin daily for control of their diabetes, only 4 were able to continue chlorpropamide therapy for more than 4 weeks. The other 7 were returned to insulin because of inadequate control; of these, 6 had disturbing side effects that, in themselves, would have been sufficient reason for discontinuing the drug. Three patients in whom control of the diabetes was lost on tolbutamide therapy were well controlled on chlorpropamide.

Side Effects

The number of side effects seemed to bear no direct relationship to the maintenance dosage of chlorpropamide. More side effects were noted in

TABLE 3
CORRELATION OF DAILY DOSAGE OF CHLORPROPAMIDE WITH SIDE EFFECTS

Dosage (gm.)	Number of cases	Side effects			
		Skin	Gastrointestinal	CNS	Other
0.25	1				
0.50	14	2	5	6	
1.0	12		3	3	
1.0	7				1*
Total		2	8	9	1*

* Deceased.

patients receiving 0.5 gm. daily than in those on higher daily dosages (TABLE 3). One patient, who had received large doses of chlorpropamide, died while receiving the drug. He suffered from arteriosclerotic and coronary artery heart disease in association with probable portal cirrhosis of the liver. Despite symptoms of anorexia and lethargy, the drug was continued until about thirty-six hours before death. At no time was hypoglycemia present. Autopsy findings showed nonspecific changes of fatty metamorphosis of the liver, cerebral atrophy, an old healed myocardial infarction, and slight aortic atherosclerosis.

Conclusions

In a small series of thirty-four cases studied thus far we have found that: Chlorpropamide was not effective in control of the brittle diabetic.

The effectiveness of chlorpropamide is not increased by large dosages, even as much as 4 gm. daily.

The toxicity of this drug apparently increases with large doses, but some patients can tolerate large daily doses and high blood levels without ill effects.

High dosage of more than 1.0 gm. daily was not associated with a higher incidence of side effects.

The drug is apparently effective in the mild diabetic.

Three patients not controlled by tolbutamide were controlled adequately by chlorpropamide.

One patient died while on large doses of chlorpropamide. The exact cause of death is unknown.

A PRELIMINARY METABOLIC AND CLINICAL EVALUATION OF CHLORPROPAMIDE

Juan M. Baertl, J. Evan Owens, George J. Gabuzda

*Department of Medicine, Western Reserve University School of Medicine at Cleveland
Metropolitan General Hospital (formerly City Hospital), Cleveland, Ohio.*

The metabolic effects and clinical efficacy of chlorpropamide, an oral hypoglycemic agent with potential therapeutic value, were evaluated in twenty-three patients with diabetes mellitus. A preliminary report of this experience with chlorpropamide is presented here.

Materials and Methods

During the course of these studies 10 patients with diabetes mellitus were selected for special study on the metabolic ward. Four of these patients, when placed under conditions of strict diet control similar to that previously recommended for them, did not demonstrate glycosuria and required no insulin. Accordingly, trials of chlorpropamide therapy were not made with these 4 patients, and they were discharged from the hospital with diet therapy alone. The other 6 were considered suitable for the metabolic studies described below. Brief descriptions of these patients are included with the data obtained for each, as presented under *Results*.

The patients were maintained on constant diabetic diets designed to meet individual requirements, each daily diet being divided into 4 feedings. The patients were weighed daily; fluid intakes were recorded, but the subjects were permitted to drink water at liberty. Twenty-four-hour outputs of urine were collected and preserved with 10 ml. of a 1:10 mixture of toluol and glacial acetic acid and refrigeration. Each daily urine volume was analyzed quantitatively for sugar content.^{1, 2}

The periods of study ranged from 18 to 62 days (average 39 days). Control periods of from 3 to 8 days preceded the administration of chlorpropamide in all patients. When it seemed permissible, insulin was withdrawn or reduced in dosage before the drug was given. The chlorpropamide* was given orally, 1 gm. daily each morning, before breakfast. One-gram doses of tolbutamide† were similarly given. Tolbutamide in daily doses greater than 1 gm. was administered in divided doses at mealtime.

Fasting blood samples were obtained frequently for sugar determinations.^{1, 2} Fasting blood levels of chlorpropamide and of tolbutamide were determined serially in some patients.³ For 3 patients, blood levels of chlorpropamide were determined several times within the 4-hour period immediately following a 1-gm. oral dose of the drug.

These studies included frequent urinalyses, complete blood counts, and tests of liver functions, including serum bilirubin concentration, alkaline phosphatase, cephalin cholesterol flocculation, and thymol turbidity. In

* Provided by Chas. Pfizer & Co., Inc., Brooklyn, New York.

† Provided by The Upjohn Company, Kalamazoo, Mich.

some patients, Bromsulphalein excretion tests also were obtained before, during, and after the administration of the hypoglycemic agent. In addition, the patients were observed closely for evidences of untoward reactions to chlorpropamide and for symptomatic hypoglycemia.

Chlorpropamide was also evaluated in 3 patients with diabetes, who were hospitalized on the general medical wards, and in 14 patients attending the diabetes clinic as outpatients. These patients were selected on the basis of their likelihood to respond to an oral hypoglycemic agent: for example, patients with stable maturity-onset type diabetes. The 3 hospitalized patients had not received insulin for at least 3 days before chlorpropamide was given. They were given 1 gm. chlorpropamide daily for at least 6 days. The outpatients were instructed to discontinue insulin and to begin chlorpropamide on the same day. Initial dose of chlorpropamide for the outpatients was 1 gm. daily for 1 week, and 0.5 gm. daily was prescribed thereafter. This therapy was continued for from 1 to 3 months (average, 9 weeks). The patients visited the outpatient department weekly. No special effort was made to ensure more adequate adherence of the patients to their diets or to other diabetic instruction previously given. They were advised to continue their diabetic regimens as in the past. These patients were aware, of course, that a change in medication was made; for example, an injection of insulin was being replaced by an oral medication and, in some cases, the frequency of required visits to the outpatient department was increased. Chlorpropamide was evaluated in the outpatients by fasting blood sugar determinations and fractional qualitative urine sugar determinations (Somogyi method). Blood sugar determinations were done almost daily in the hospitalized patients and, usually, weekly in the outpatients. In addition, daily quantitative glycosuria was determined for the patients in the hospital. Blood chlorpropamide levels were determined at weekly intervals in some of the outpatients. Urinalyses, hemograms, blood urea nitrogen concentration, and liver function tests were also done at intervals for these patients.

Results

Metabolic studies. Since it was not possible to carry out identical protocols for the six patients studied on the metabolic ward, the data for each of these patients are presented separately.

Patient J. M., a 42-year-old Negro male, diabetic for 1 year, was admitted to the hospital because of diabetic acidosis. Of his own accord he had stopped taking insulin 6 months prior to admission. The acidosis was treated, and he required 15 U. NPH insulin daily for 2 weeks preceding the metabolic study. The results of this study are presented in FIGURE 1. During the initial 7-day period of metabolic study, this patient's diabetes was well controlled with a 2500-cal. diet and 15 U. of NPH insulin daily. Insulin was then discontinued. During the 6 days without insulin, glycosuria increased from less than 5 gm. to about 30 gm. daily, and fasting blood sugar levels increased from 160 mg. to 210 mg. per cent. Then 1 gm. chlorpropamide was given orally daily. In association with chlorpropamide therapy glycosuria and fasting blood sugar levels promptly decreased.

Twenty-four hours after the initial 1-gm. dose had been given, fasting blood sugar level had decreased from 210 mg. to 164 mg. per cent and, after several days, ranged between 110 and 132 mg. per cent. Glycosuria was less than 5 gm. daily, and remained so even after the dose of chlorpropamide was decreased to 0.5 gm. daily on the tenth day of therapy. Upon discharge from the hospital a similar diet and 0.5 gm. chlorpropamide were prescribed for this patient, who has been seen in the outpatient clinic at regular intervals and has maintained excellent control of diabetes under this regimen during

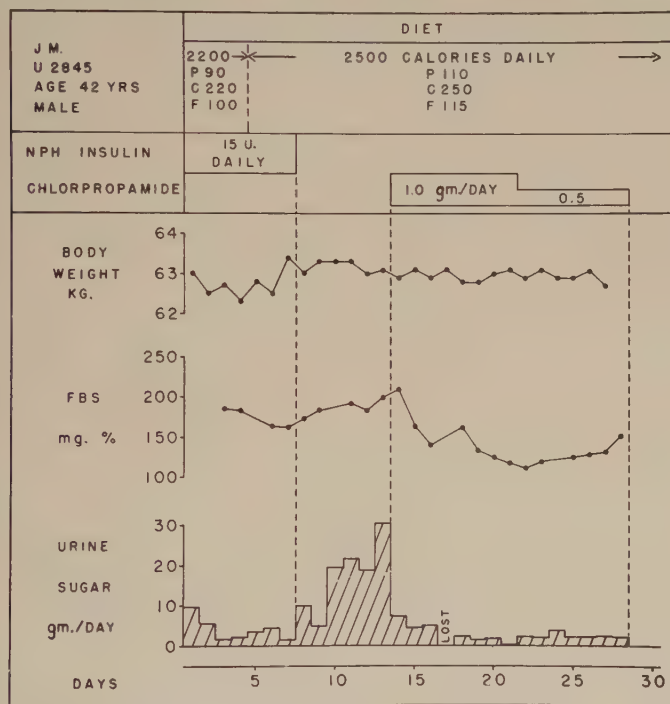


FIGURE 1

the 6-month period to date. The patient demonstrated a prompt and adequate hypoglycemic response to chlorpropamide administered orally, and it replaced insulin in his diabetic regimen.

Patient A. M., a 60-year-old Negro female with stable, easily controlled diabetes of 10 years' duration, required 30 U. of NPH insulin daily. This patient had a past history of repeated episodes of pancreatitis, and it seemed probable that the diabetes was secondary to chronic pancreatitis. She was being treated in the hospital for pulmonary tuberculosis and received isoniazid and para-aminosalicylate while this study was in progress. Insulin was discontinued 1 week before metabolic study was initiated. The findings in patient A. M. are presented in FIGURE 2. During the first 3 days of study, this patient was given no insulin but was maintained on a constant 2000-cal.

diabetic diet. Glycosuria increased from 68 gm. to 94 gm. daily, fasting blood sugar levels increased from 194 mg. to 360 mg. per cent, and the patient lost weight. Chlorpropamide, 1 gm. daily, was then given orally. After 48 hours, glycosuria had decreased to 54 gm. daily, fasting blood sugar level had decreased to 228 mg. per cent, and body weight was maintained. Chlorpropamide was given for 6 days, following which fasting blood sugar levels

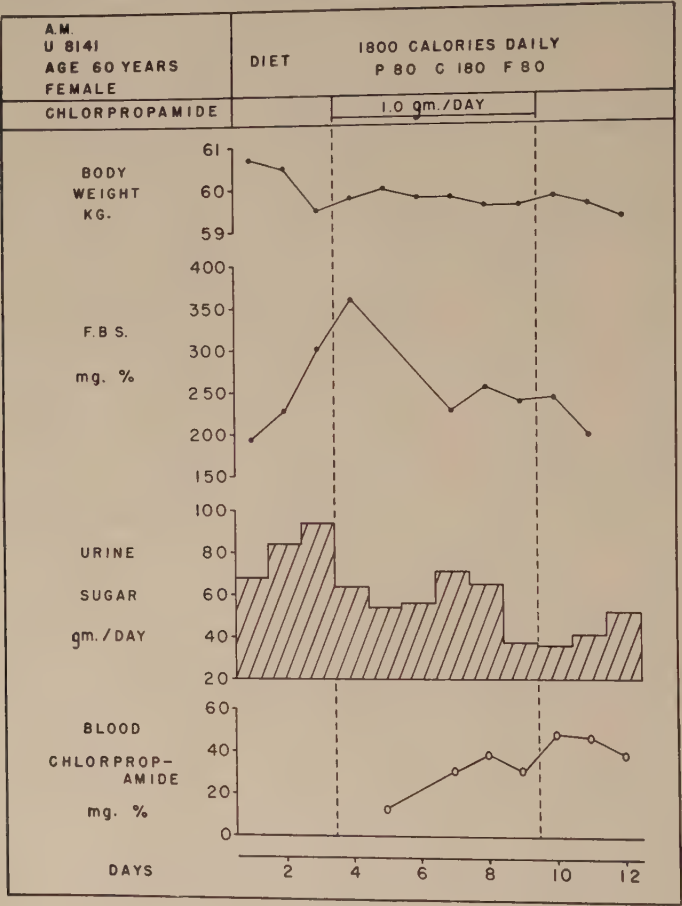


FIGURE 2

were about 200 mg. per cent and glycosuria 35 to 40 gm. daily. Although this patient demonstrated a hypoglycemic response to chlorpropamide, glycosuria was not decreased sufficiently to warrant considering diabetic control as excellent. Fasting blood levels of chlorpropamide were determined in this patient during and after the administration of the drug. These levels increased progressively during therapy to 54 mg. per cent (FIGURES 2, 3).

Patient M. L., a 62-year-old Negro female, had stable diabetes of 12 years duration that was not well controlled with 10 U. NPH insulin daily. She

was admitted to hospital for regulation of diabetes. This patient also had arteriosclerotic heart disease and took digitalis daily. The initial 5-day control regimen for M. L. (FIGURE 4) consisted of a 2000-cal. diet and 10 U. of NPH insulin daily. During this period, fasting blood sugar levels ranged from 254 to 304 mg. per cent, and glycosuria from 44 to 104 gm. Beginning on the sixth day, 1 gm. chlorpropamide was given daily in addition to the insulin, and these medications were continued for 12 days. Glycosuria and fasting blood sugar levels gradually decreased to less than 10 gm. daily and approximately 100 mg. per cent, respectively. Insulin was then discontinued but chlorpropamide continued for an additional 6 days, during which

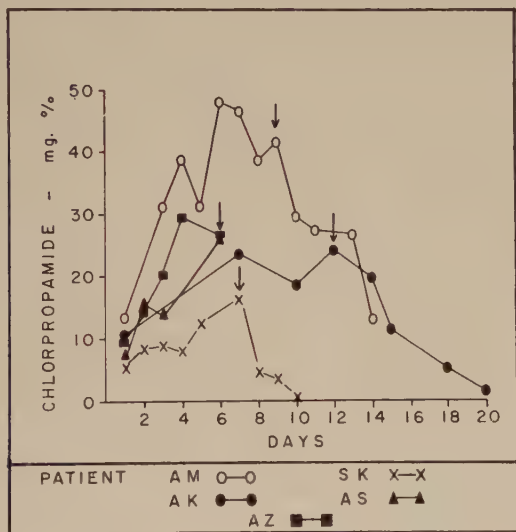


FIGURE 3. Blood levels after oral administration of 1 gm. per day of chlorpropamide. Arrows indicate the days on which chlorpropamide was discontinued.

excellent control of diabetes was sustained. Discharged on the same program, the patient has maintained the same excellent control of diabetes with chlorpropamide 0.5 gm. daily for a 4-month period, to date. This patient demonstrated a slow progressive response to chlorpropamide, with the eventual replacement of insulin by this agent. The apparent slow response to chlorpropamide makes this study somewhat difficult to interpret but, since it had been known that this patient had diabetes for 12 years and had required insulin during this time, this case is considered a positive response to chlorpropamide.

Patient A. K. (FIGURE 5), a white female 63 years of age, was found to have diabetes following a recent admission to the hospital for thrombophlebitis of the left lower leg. She also demonstrated senile dementia, but was able to cooperate in the adequate collection of urine specimens. Prior to the initiation of study she had persisting glycosuria of about 20 gm. daily although she was given a prescribed diet and 40 U. of NPH insulin daily.

This patient had about 25 gm. glycosuria daily, and fasting blood sugar levels of 280 mg. per cent during the 2 initial days of observation on the metabolic ward while she was consuming a 2000-cal. diet and receiving 40 U. of NPH insulin daily. Two grams of tolbutamide, given orally, in addition to the insulin, produced a prompt decrease in glycosuria to less than 5 gm. daily and in fasting blood sugar to 170 mg. per cent; this was apparent after the first day of therapy. Discontinuance of insulin was followed by transitory increases in urine and blood sugar levels, but control of diabetes continued

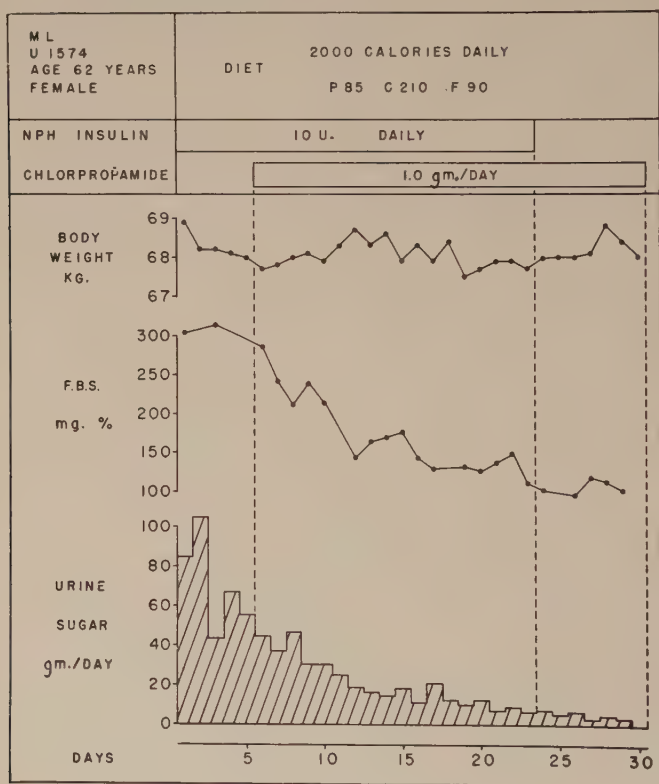


FIGURE 4

to be adequate. After 11 days of tolbutamide administration, this agent was discontinued, following which glycosuria and hyperglycemia increased, reaching values of 30 gm. daily and 250 to 280 mg. per cent, respectively, after 8 days. One gram of chlorpropamide was then given orally daily for 13 days. Chlorpropamide administration resulted in decreased hyperglycemia and glycosuria, although these responses occurred more slowly than they did following the tolbutamide previously administered. Although the hypoglycemic responses were comparable, the glycosuria was not decreased as much with chlorpropamide as it was with tolbutamide. After chlor-

propamide was discontinued, glycosuria and hyperglycemia increased during a 9-day control period. During a final period of study, 1 gm. of tolbutamide was given daily. As in the earlier period of tolbutamide administration, the effect of this agent on blood sugar and glycosuria was prompt. The urine sugar excretion decreased from about 30 gm. daily to less than 10 gm. on the first day this drug was used. Maximal blood sugar response was noted 48 hours after tolbutamide therapy was started, by which time levels

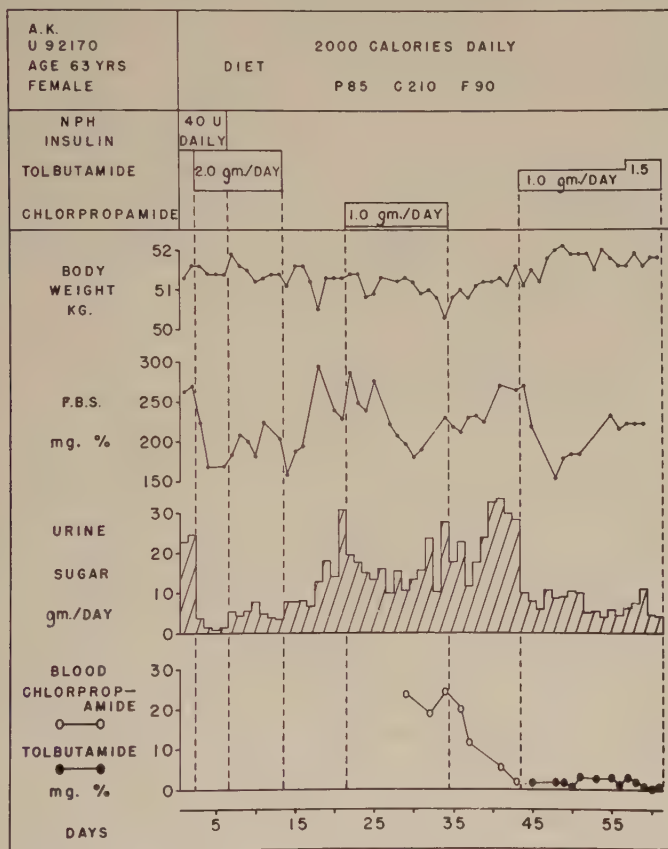


FIGURE 5

had decreased from about 265 mg. per cent to 154 mg. per cent. Blood sugar then increased gradually to approximately 230 mg. per cent, at which level it became stable; glycosuria averaged less than 10 gm. daily. Increasing the dose of tolbutamide from 1.0 to 1.5 gm. had no appreciable effect on blood or urine sugar levels.

Serial fasting blood levels of chlorpropamide and tolbutamide were done for this patient (FIGURE 5). During the last 6 days of chlorpropamide therapy, blood levels of this agent ranged from 18 to 25 mg. per cent. After

chlorpropamide was discontinued, the drug gradually disappeared from the blood. Approximately 7 days were required for its complete disappearance. Only small amounts of tolbutamide were detectable in the fasting blood samples obtained during the administration of this agent.

Both chlorpropamide and tolbutamide demonstrated hypoglycemic and aglycosuric effects in patient A. K. The response to tolbutamide was more prompt and more striking than the response to chlorpropamide.

Two additional patients (E. H. and S. K.) who were studied on the metabolic ward for thirty-six and sixty days, respectively, failed to demonstrate any significant responses to the oral administration of chlorpropamide or of tolbutamide. Brief résumés of these patients follow.

Patient E. H., a 46-year-old man, had very labile diabetes. He had experienced frequent episodes of acidosis and hypoglycemia, many of which required hospitalization, during the 10 years since the diagnosis of diabetes mellitus was made. His usual daily insulin dosage was 40 U. of PZI insulin each morning and 30 to 50 U. of regular insulin during the day. He was not well controlled despite many efforts in the hospital to accomplish an adequate regimen. His basal control regimen consisted of a 1900-cal. diet and insulin (as indicated above). Following a 12-day control period, he was given, in addition, 1 gm. chlorpropamide daily for 22 days. This period of chlorpropamide administration was followed by the basal regimen for 14 days. Then 2 gm. tolbutamide was given orally each day for 13 days. There was no effect attributable to either of these hypoglycemic agents.

Patient S. K., a lean man 47 years of age, was admitted to hospital in diabetic acidosis as a newly discovered diabetic. He responded promptly to treatment for diabetic acidosis, and then his diabetes was readily controlled with a 2500-cal. diet and 35 U. of NPH insulin daily. After 9 days in hospital, he was transferred from a general medical ward to the metabolic ward for a trial of hypoglycemic agents. During the initial 8-day period of study, adequate control of diabetes was easily maintained with the diet and insulin dosage indicated. Fasting blood sugar levels ranged from 125 to 155 mg. per cent and glycosuria was less than 5 gm. per day. For the next 4 days, insulin was not given, after which fasting blood sugar increased to 340 mg. per cent and glycosuria to approximately 115 gm. daily. One gram of chlorpropamide was then given orally for 6 days. The drug had no effect upon the hyperglycemia or urinary sugar excretion. Following a 3-day control period without insulin, tolbutamide was given orally (1 gm., 3 gm., and 4 gm. orally daily for 3 successive periods of 3 days each). Tolbutamide was also without therapeutic effect; the patient was again given insulin and excellent control of diabetes was readily achieved.

Fasting blood levels of chlorpropamide and of tolbutamide were obtained for S. K. (FIGURE 3). Levels during chlorpropamide therapy ranged from 6 to 17 mg. per cent. After tolbutamide had been given for 9 days in dosages increasing from 1 gm. to 4 gm. daily, 14 mg. per cent of tolbutamide was detectable in fasting blood specimens. The failures of response in this patient could not therefore be attributable to a failure of absorption of the hypoglycemic agents. In S. K., approximately 3 days were required for

the complete disappearance of chlorpropamide from the blood after cessation of therapy.

Chlorpropamide blood levels. In some patients, chlorpropamide blood levels were determined at intervals during the 4-hour period following a 1-gm. oral dose. These data are presented in FIGURE 6. The average blood level was about 9 mg. per cent after 1 hour, and 12 mg. per cent 4 hours after its ingestion, indicating prompt absorption of this agent.

The serial chlorpropamide blood levels obtained during the studies of patients on the metabolic ward are presented compositely in FIGURE 3. The patients received 1 gram of chlorpropamide daily for the time indicated

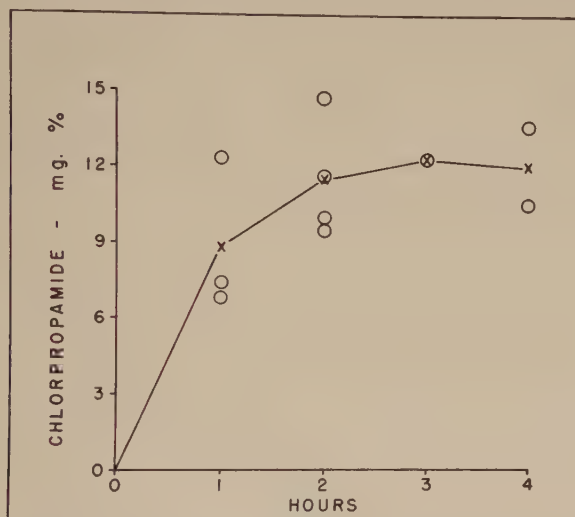


FIGURE 6. Blood levels after oral administration of 1 gm. chlorpropamide. Curve from X to X indicates the average value for each interval following chlorpropamide administration.

on the chart. Blood levels of the drug were cumulative at this dosage. This relatively small group of patients demonstrated considerable variation in blood level attained with comparable doses of the drug. The rates of disappearance of chlorpropamide from the blood after therapy varied from 3 to 7 days in these patients.

The blood levels of chlorpropamide determined at weekly intervals in some of the patients attending the outpatient clinic are presented in TABLE 1. Although these data are admittedly meager, they suggest that maintenance doses of 0.5 gm. daily of chlorpropamide may be adequate to maintain blood levels of approximately 20 mg. per cent.

Clinical studies. Three patients hospitalized on the general medical service were given clinical trials with chlorpropamide. One of these patients (a 60-year-old, female, newly discovered diabetic) responded adequately to chlorpropamide, in a dosage of 1 gm. daily for 6 days, with a decrease in fasting blood sugar to levels below 150 mg. per cent; she became essentially

aglycosuric. Continued on a maintenance dose of 0.5 gm. daily, she has been observed in the outpatient clinic for 2 months, during which time diabetic control has been sustained. The other 2 patients of this group (a 70-year-old female and a 46-year-old male), each of whom required 20 U. of NPH insulin for control of diabetes, did not respond adequately to 1 gm. chlorpropamide given daily for 1 week; they were returned to insulin therapy.

The fourteen outpatients selected for study ranged in age from thirty to eighty-three years, with an average age of sixty-one years. Diabetes had been established for periods of from six months to twenty-eight years. The average duration of diabetes was ten years. In all patients the diabetes was the stable maturity-onset type.

TABLE 1
CHLORPROPAMIDE BLOOD LEVELS IN DIABETIC OUTPATIENTS

Weeks of therapy	Daily dose (gm.)	No. of patients	Level, mg. per cent	
			Range	Average
1	1.0	3	30-34	32
3	0.5	6	14-33	23
5	0.5	3	13-24	18
7	0.5	2	18-20	19
9	0.5	2	20-23	22

Three of the patients had been treated with diet therapy only; the remainder had been treated with diet and from 10 to 35 U. insulin. The average insulin dose was 25 U. daily.

All patients were given similar doses of chlorpropamide: 1 gm. per day for the first week followed by one-half gm. per day as a maintenance dose thereafter.

An adequate clinical response to this drug was established as one exhibiting aglycosuric fractional urines, lack of subjective symptoms, maintenance of body weight, and reduction in fasting blood sugars—preferably below 150 mg./100 cc.

In 12 patients the diabetes was adequately controlled with a single daily dose of chlorpropamide as judged by the above criteria. At present, these patients continue to be treated with diet and chlorpropamide therapy. Seven showed an average reduction in the fasting blood sugar of 105 mg. per cent from previous levels and continued to be maintained at levels below 150 mg. per cent. Seven others showed no appreciable change in fasting blood sugar levels; however, most of them had been well controlled on their previous therapy.

Two patients were not adequately controlled with chlorpropamide. One, a 63-year-old female with mild diabetes of 21 years' duration, previously controlled with 15 U. of insulin, did not respond after 4 weeks of treatment.

and was returned to insulin therapy. This patient was the only one to express subjective symptoms to chlorpropamide therapy. She complained of epigastric distress during treatment, which disappeared when chlorpropamide was discontinued; she was being treated simultaneously for a chronic duodenal ulcer.

The other patient, a 45-year-old male with mild diabetes of 3 years' duration, previously controlled with 25 U. of insulin, did not respond adequately after 4 weeks of treatment with chlorpropamide; insulin therapy was resumed.

Chlorpropamide appeared successful in controlling the diabetes in 12 of 14 of this group of selected outpatients, most of whom were in the older age group with stable diabetes previously treated with diet therapy alone or in conjunction with moderate doses of insulin. Control has been maintained for as long as 6 months.

Toxicity. With the exception of the patient mentioned above, who experienced epigastric distress, there were no subjective symptoms of toxicity nor were there laboratory evidences of disturbances of renal, hepatic, or hematological function in any of the patients studied. Symptomatic hypoglycemia did not occur.

Discussion

Chlorpropamide was an effective oral hypoglycemic agent when administered orally to most of the patients (with diabetes mellitus) studied. As with other oral hypoglycemic agents, patients with stable, maturity-onset type diabetes demonstrated more adequate responses than relatively young patients with labile diabetes. There were exceptions to this, however, in that two of the outpatients studied, who were judged to fulfill the accepted indications for therapy with an oral agent, failed to respond to chlorpropamide. Furthermore, the patterns of response to chlorpropamide, as evaluated in the six patients studied on the metabolic ward, were quite variable. One patient demonstrated a prompt and adequate response (J. M., FIGURE 1); another, a delayed response (M. L., FIGURE 4); others showed significant but inadequate responses (A. M., FIGURE 2; A. K., FIGURE 5). The reasons for this variation are not apparent; they did not relate to the blood level of chlorpropamide. Although blood levels varied considerably among patients given similar doses of the drug (FIGURE 3), blood level did not correlate with the adequacy of diabetic control attained with chlorpropamide.

Four patients who either failed to respond or demonstrated inadequate responses to chlorpropamide were subsequently given tolbutamide. Three of these also failed to demonstrate responses to tolbutamide (E. H., S. K., A. M.). A fourth patient (A. K., FIGURE 5) demonstrated more prompt and pronounced effects on blood and urine sugar when she was given tolbutamide than when she was given chlorpropamide in comparable dosage.

Studies of the level of chlorpropamide in the blood following its oral administration indicated that it is promptly absorbed and slowly excreted. Three to seven days were required after cessation of therapy before the drug disappeared from the blood. In this respect it differs from tolbutamide, which is promptly absorbed and more rapidly removed from the blood. With 1-gm.

daily doses of chlorpropamide, blood levels of the drug progressively increased in some patients. Those who demonstrated adequate responses to 1.0 gm. of chlorpropamide given daily for about 1 week subsequently sustained adequate control of diabetes with a continuing dose of 0.5 gm. daily. Preliminary data suggest that, with maintenance doses of 0.5 gm. daily (FIGURE 3), chlorpropamide blood levels can be sustained at about 20 mg. per cent. Whether this is an optimal blood level cannot be stated; it is possible that doses less than 0.5 gm. daily might have been satisfactory for maintenance in some patients. On the other hand, some patients might have demonstrated more adequate responses had larger doses been employed (S. K., FIGURE 3). In the studies reported here, no effort was directed to establish either the minimally effective dose of chlorpropamide or the maximum safe dose.

An important consideration in the acceptance of a drug for clinical use is its freedom from untoward effects. Although no serious untoward symptoms or laboratory findings resulted from the administration of chlorpropamide to the twenty-three patients studied, conclusions regarding toxicity in man must be based on a much larger experience than is reported here.

Summary

The clinical and metabolic effects of chlorpropamide administered orally were evaluated in 23 patients with diabetes mellitus. Six of these were studied under carefully controlled conditions on a metabolic ward. The other 17 were observed on the general medical wards (3 patients), or in the outpatient clinic (14 patients).

Chlorpropamide given orally (1 gm. daily) decreased glycosuria and fasting blood sugar levels in 4 of the 6 patients studied on the metabolic ward. Adequate clinical responses were obtained in 2 stable diabetics for whom insulin was completely replaced. Inadequate responses were found in 2 other stable diabetics who had required 40 U. of NPH insulin daily. One of the patients who was inadequately controlled with chlorpropamide demonstrated more prompt and adequate decreases in glycosuria following identical doses of tolbutamide. The other 2 patients (a lean 47-year-old man with newly discovered diabetes and a 46-year-old man with very labile diabetes) failed to demonstrate responses to chlorpropamide or to tolbutamide.

Serial blood levels indicated that chlorpropamide was absorbed promptly but excreted slowly. Three to seven days elapsed before it disappeared from the blood following cessation of therapy. Blood levels attained with the same dose (1 gm. daily) of chlorpropamide demonstrated considerable variation in the patients studied.

Clinical trials of chlorpropamide were conducted in 17 patients who were selected because of their likelihood to respond to an oral hypoglycemic agent. In 13 of these patients the diabetes was adequately controlled for as long as 6 months with a single daily oral dose of chlorpropamide (1 gm. daily for 1 week, and then 0.5 gm. daily). With this dosage schedule, chlorpropamide blood levels of about 20 mg. per cent were maintained.

With the exception of epigastric distress in one patient, there were no

untoward clinical manifestations attributable to chlorpropamide. Symptomatic hypoglycemia was not observed. Serial urinalyses, hemograms, and tests of hepatic and renal functions were not altered during the periods of study.

Acknowledgment

The chlorpropamide and tolbutamide blood levels were determined by arrangement with Max Miller, Western Reserve University School of Medicine. These determinations were done in his laboratories by Naomi F. Nickerson. The authors are grateful for this contribution to the study.

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CHLORPROPAMIDE: A NEW SULFONYLUREA STUDIED IN THE TREATMENT OF THIRTY DIABETIC PATIENTS

Alfredo Piazza and Walter Chanamé

Metabolic Clinic, Hospital Obrero de Lima, Lima, Peru

A few years ago insulin was the only drug available for the control of diabetes. In 1955, German authors^{1, 2} reported the results of the treatment of diabetic patients with carbutamide, an oral sulfa drug with a marked hypoglycemic effect. Shortly thereafter a new compound known as tolbutamide, closely related to the first, was also employed.³ Loubatières reviewed in a complete and concise presentation the history and development of these compounds.⁴

We have used both drugs since 1955 in a great number of cases. Usually, overweight patients more than 40 years of age, with diabetes of short duration and without previous insulin therapy, have been successfully controlled with these oral compounds. Sulfa drugs have not been effective in juvenile diabetics or in thin mature individuals who have received insulin for several years.

Chlorpropamide, a recently developed oral drug closely related to tolbutamide, has also shown a hypoglycemic effect in animal studies. The purpose of this paper is to report our experience during the last eight months in administering this compound to diabetic patients.

Methods

Chlorpropamide was given to 30 patients (4 females and 26 males) observed at the Metabolic Clinic. The ages of these patients ranged from 17 to 70 years, but most were between 40 and 65 years. All were Mestee (white and Indian) except 3 Negroes. Four cases were classified as severe diabetes, 20 as moderately severe, and 6 as mild. The disease had started from a few months to 20 years before but, in most cases, it had existed 1 to 8 years. There was 1 case of diabetes (No. 29) due to tumoral invasion of the pancreas.

Twenty-four patients were on insulin therapy or had received this drug for some time. Twenty-four were taking or had taken oral drugs: 12, carbutamide and 12, tolbutamide. Five had not had any previous therapy. All had been admitted before to the hospital for metabolic studies and treatment and then observed for months or years at the Metabolic Clinic as outpatients. Most were readmitted for several days to start the new treatment. TABLE 1 contains all pertinent data concerning each of the patients studied.

A careful physical examination was performed, blood sugar⁵ was determined at least twice a week, and urine was examined for sugar four times a day before meals. The following tests were also done: complete urinalysis, blood count and differential, blood urea, creatinine and bilirubin, thymol turbidity, colloidal gold, cephalin-cholesterol flocculation, serology, and sedimentation rate.

The patients were on a standard diabetic diet. Oral antidiabetics were

discontinued for 1 or 2 weeks, and insulin injections were discontinued for 48 hours. A chlorpropamide tolerance test was then performed. A fasting venous blood sample having been obtained, 3 gm. of the drug was given orally; 4 more hourly blood specimens were then taken. Blood sugar was determined according to Folin's micromethod.

TABLE 1
DATA ON THIRTY PATIENTS TREATED WITH CHLORPROPAMIDE

Case no.	Age, years	Sex	Weight, per cent	Severity of diabetes*	Duration, years	Insulin	Carbutamide	Tolbutamide	Chlorpropamide tolerance test			Blood sugar reduction, percentages	Average doses per day	Length of treatment, days	Results†
									First hour	Second hour	Fourth hour				
1	56	M	-5	+	1	0	0	0					0.125	90	E
2	42	F	+38	++	4	+	0	+	190	145	90	53	1	7	G
3	51	F	+38	++	7	+	+	+	185	185	134	28	1.250	10	B†
4	55	M	+12	++	7	+	0	0	178	155	123	31	0.750	98	E
5	49	M	+22	++	6	+	+	0	95	100	88	8	0.500	180	E
6	53	M	+11	+	1	0	0	0	356	270	258	28	0.250	150	E
7	34	M	+15	+	4	+	0	0	107	89	86	20	0.250	105	E
8	17	M	-6	+	1	+	0	0	80	50	30	63	1	105	E
9	55	M	-28	+++	12	+	0	+	280	280	290	+4			
10	68	M	-5	++	18	+	+	0	130	83	57	56	0.250	197	E
11	40	M	+19	++	4	+	+	0					0.875	119	E
12	21	M	-13	+++	4	+	0	0	215	215	215	0			
13	63	M	+11	++	8	+	0	+	180	160	148	18	1.500	63	F
14	46	F	+5	++	3	+	0	+	160	150	129	20	1	28	G
15	65	M	-14	++	8	+	0	+	300	320	295	2			
16	31	M	+8	+	1	0	0	0	228	198	170	26	0.250	111	E
17	53	M	+16	++	4	+	+	+	160	125	93	42	0.500	56	E
18	70	M	+19	++	10	+	+	0					0.250	191	E
19	57	M	+7	++	2	+	0	+	142	85	95	33	0.250	185	E
20	67	M	-20	++	8	+	+	0					0.125	200	E
21	57	M	-14	++	1	+	+	+	222	216	178	20	2	30	E
22	32	F	+19	++	10	+	+	0	190	190	120	37	1.500	213	G
23	46	M	+32	++	9	+	+	0	128	120	110	14	0.750	200	E
24	42	M	-1	+++	5	+	0	+	225	240	230	+2			
25	35	M	-11	+	1	0	0	0	170	152	112	34	0.500	119	E
26	65	M	-24	++	20	+	+	+	169	107	86	49	1.250	70	G
27	54	M	-5	+++	5	+	0	0	175	210	215	+23			
28	52	M	+18	++	1	0	0	0	194	170	116	40	0.500	10	E
29		M			6	+	+	0	180	155	140	22	2.250	22	G
30	48	M	+25	++	9	+	+	+	230	187	160	31	1	8	E

* Key: +, mild; ++, moderately severe; +++, severe.

† Treatment discontinued because of intolerance.

‡ Key: E, excellent; G, good; F, fair; B, bad.

This initial test dose was followed, in most of the cases, by 1 gm. daily, taken one-half after breakfast and one-half after dinner. This dosage was gradually reduced according to the blood sugar level. Control was continued every 2 or 3 weeks at the outpatient clinic. Sugar was checked in blood and

urine each time. Periodically, hepatic, renal, and hematological tests also were performed.

Results

Twenty-four of 30 patients studied have been adequately controlled with chlorpropamide and a standard diabetic diet for as long as 7 months. Fourteen had a blood sugar reduction of 25 per cent or more when the tolerance test was performed. FIGURE 1 illustrates a response typical of patients in the group. Four patients had reductions of 20 per cent, and 1 each of 18, 14, and 8 per cent. The average maintenance dose was 600 mg. per day, varying between 125 and 2000 mg.

One patient (No. 21), who had a 20 per cent blood sugar reduction at the tolerance test, could not be controlled with chlorpropamide alone and

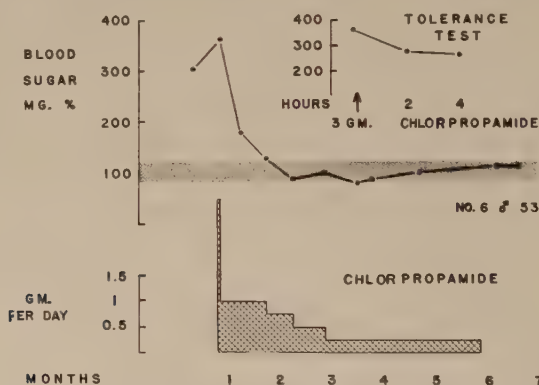


FIGURE 1. Chart of a 53-year-old male (patient No. 6) without previous therapy who had a 28 per cent reduction in blood sugar during the chlorpropamide tolerance test. Normal values were maintained during 6 months of therapy. No increase in the blood sugar level was observed 1 month after discontinuing the drug.

required insulin. Nevertheless, after several days of combined therapy, it was found that insulin could be discontinued, leaving the patient on the oral drug alone (FIGURE 2). It is interesting to note that this patient had also had a 21 per cent blood sugar reduction when 3 gm. of tolbutamide was given; however, control could not be achieved with this drug alone.

Another patient (No. 13) had an 18 per cent sugar reduction, but only a partial control was obtained. Symptoms disappeared, but blood sugar level was brought no lower than 150 mg. per cent.

The tolerance test was negative in five patients (Nos. 9, 12, 15, 24, and 27), showing either no reduction of the initial blood sugar level or a rise. All had shown the same lack of response to carbutamide and tolbutamide and all had a diabetes of long duration and had been on insulin therapy for more than four years.

Chlorpropamide, 1.5 gm. daily, was given in conjunction with insulin in 3 of these cases. A mild favorable effect was demonstrated in 2 (Nos. 12 and 15) and none in the third (No. 27).

Chlorpropamide was tolerated very well by most of the patients. There were two (Nos. 13 and 20) who complained of mild dizziness (like drunkenness) not related to hypoglycemia and lasting a few days. During this time they were able to continue their work. The discomfort gradually disappeared, and the treatment was not discontinued.

Only 1 patient (No. 3) showed marked intolerance. She had had a 28 per cent blood sugar reduction on the initial test and did not complain of any immediate discomfort at that time. Nevertheless, she developed nausea,

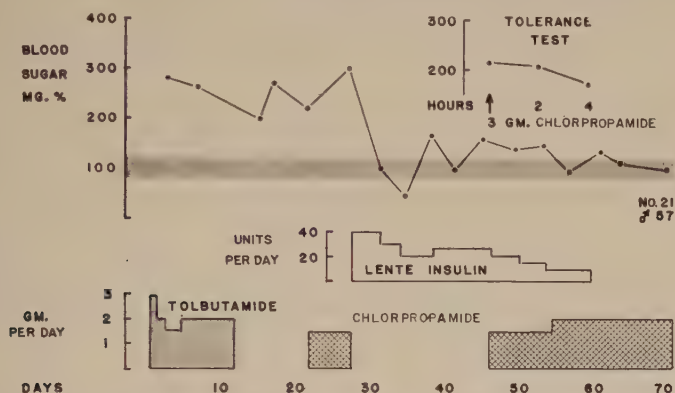


FIGURE 2. Chart of a 57-year-old Negro male (patient No. 21) who had a 21 per cent reduction in blood sugar during the tolbutamide tolerance test and a 20 per cent reduction with chlorpropamide. Blood sugar could not be brought to normal after 12 days' treatment with tolbutamide (2 gm. per day). Chlorpropamide (1.5 gm. per day) was given for 8 days without success. Forty U. per day of lente insulin was then injected; blood sugar was lowered rapidly to normal levels. After 17 days of good carbohydrate metabolism, chlorpropamide was resumed, and insulin was gradually reduced and finally discontinued.

vomiting, epigastric burn, diarrhea, dizziness, and headache after a few days of treatment at a dosage of 1.5 gm. daily. Symptoms stopped when the drug was discontinued, but reappeared each time an attempt was made to resume the treatment. She had tolerated tolbutamide well, but the drug did not control her diabetes.

In none of the thirty cases were skin reactions or hematological, renal, or hepatic changes observed.

No particular reaction was noticed in four patients (Nos. 5, 19, 20, and 23) who had pulmonary tuberculosis. One, with a partial gastrectomy performed 3 years before because of a perforated duodenal ulcer, had an excellent evolution. He is taking 250 mg. every other day and has been observed for 200 days.

No changes in the blood pressure level occurred. Two patients (Nos. 6 and 17) with high blood pressure and normal renal function tests had to continue taking hypotensive drugs. Two others with arteriosclerosis and high blood pressure did not show any change (Nos. 10 and 26). One (No. 10), who had renal insufficiency (urea, 100 mg. per cent), was maintained on a low protein diet during 197 days of therapy. In addition to an excellent carbo-

hydrate balance, a slight decrease in blood urea level was achieved (urea, 80 mg. per cent). This patient is now taking 250 mg. per day.

Discussion

Chlorpropamide seems to have the same mode of action as carbutamide and tolbutamide, but it seems to be more powerful, most patients requiring a lower dosage.

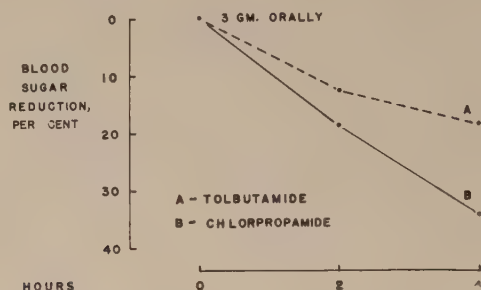


FIGURE 3. Comparative average blood sugar reduction (percentage) in nine patients. Reduction was 17.5 per cent for tolbutamide and 35.3 per cent for chlorpropamide.

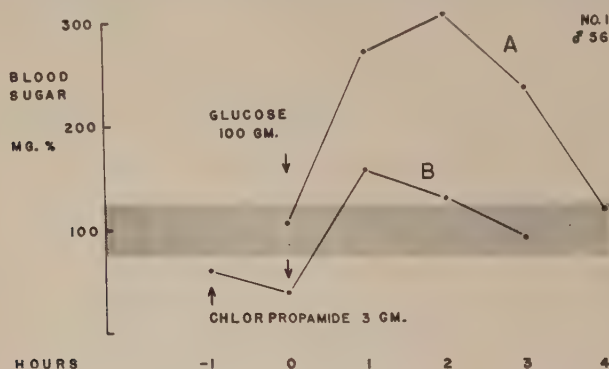


FIGURE 4. An abnormal oral 100-gm. glucose tolerance test (patient No. 1) became almost normal when repeated 1 hour after administration of 3 gm. of chlorpropamide.

When a 4-hour tolbutamide tolerance test was performed on 9 patients, an average blood sugar reduction of 17.5 per cent was found. A chlorpropamide tolerance test performed in the same group showed a reduction of 35.3 per cent (FIGURE 3). Four patients previously controlled with an average daily dose of 1375 mg. of tolbutamide required only 1250 mg. of chlorpropamide. Eight patients who were on an average of 1000 mg. of carbutamide required only 744 mg. of chlorpropamide.

Five patients (Nos. 2, 5, 17, 21, and 30), adequately controlled with chlorpropamide, previously had not been balanced with other oral drugs alone, requiring insulin also.

An abnormal glucose tolerance test in 1 patient (No. 1) showed an almost

normal reaction when repeated 1 hour after the patient had taken 3 gm. of chlorpropamide (FIGURE 4).

It is interesting to note that a seventeen-year-old patient (No. 8) has been very well controlled with this drug.

Summary

The activity of chlorpropamide in 30 diabetic patients formerly treated with insulin, carbutamide, or tolbutamide has been studied. Venous blood sugar levels during the 4 hours following oral administration of 3 gm. of chlorpropamide were previously determined. The drug was administered to 27 of these patients for as long as 7 months. The percentage of blood sugar reduction obtained in the initial test is related to the results of the treatment. The curves plotted from these data are compared with similar tests that used tolbutamide.

The drug was well tolerated. Its mode of action seems to be similar to that of previously used sulfa drugs. Nevertheless, chlorpropamide seems to be more powerful and, in some cases, more effective; a lower dosage was required by most patients. It was possible to keep in good balance some cases that had not responded adequately to other oral drugs. No skin reactions and no hematological, renal, or hepatic changes were observed.

Acknowledgment

The authors are indebted to German Stiglich and Luis Espejo for their cooperation. The valuable technical assistance of Laura Garcia and Albuina Muñoz is acknowledged. The chlorpropamide used was furnished by Chas. Pfizer & Co., Inc., Brooklyn, N. Y.

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PRELIMINARY REPORT ON THE TREATMENT OF DIABETES MELLITUS WITH CHLORPROPAMIDE*

Miguel Ruiz-Guía

Research Department, Monte Carmelo Medical Clinic, Caracas, Venezuela

Chlorpropamide was made available to us early in July 1958. To initiate the investigation, 113 unselected diabetic patients were chosen. Twenty-five of these were rejected because they showed satisfactory control with diet alone, and 6 were considered not suitable for exposure to chlorpropamide because of severe metabolic nephropathy. In 3 cases treatment was suspended because of intercurrent conditions not related to the use of the drug under investigation and, in 27 cases, the study was discontinued for non-medical reasons. Of the original group, 52 were left to be considered in this study which, because of the small number of cases that have been studied thus far and the shortness of the observation periods (5 to 36 days), should be regarded as a preliminary report.

Material and Methods

All patients were treated on an ambulatory basis; they and their relatives received careful instructions regarding such aspects as diet and the performance of urine test for sugar. On the days the blood sugar was determined the patients were admitted to the outpatient dining room of the Monte Carmelo Medical Clinic, where the meals were served. Every patient under study was submitted to the following controls: (1) medical consultation at least once a week; (2) determination of venous blood sugar (Folin-Wu method), while fasting and between meals; (3) routine urinalysis; and (4) hematological studies (red and white cells, platelet counts, hemoglobin and hematocrit determinations, and differential white counts), as well as blood urea nitrogen determinations.

Generally speaking, the diets prescribed had the following approximate composition for a 24-hour period: 200 to 250 gm. of carbohydrates, 1 to 1.5 gm. of protein per kilogram of body weight, and fat to complete the caloric requirements. Because of food habits in Venezuela, the diet contained liberal amounts of foods rich in easily available carbohydrates (rice, bread, pastry, cornbread, potatoes, and tubers such as yucca and ocumo). For the same reason, the distribution of the carbohydrates of the diet was, in general, as follows: 20 per cent at breakfast, 5 per cent at mid-morning, 40 per cent at lunch, 10 per cent at 4 P.M., and 25 per cent at dinner. In those patients in which it was advisable not to omit insulin completely, only the regular type of this hormone was kept in use, administered to the patients 2 to 3 times a day according to the amount of glycosuria present before breakfast, lunch, or dinner (or both the latter). Regarding chlorpropamide in most cases we used rather small doses, trying to determine the smallest

* The study reported here was supported in part by a grant from the Pfizer Corporation of Venezuela, supplier of the chlorpropamide used.

active one necessary to control the patients receiving it (TABLE 1). In a few cases with severe diabetes we prescribed larger doses, but in no instance was any dose more than 500 mg., 3 times a day. We do not know whether the number of patients showing satisfactory response would have been larger if we had used larger doses or if we had given the drug for a longer period of time, or both. It is to be noted that in 3 cases it seemed that a better action of the drug upon the blood sugar was obtained if chlorpropamide was given 1 hour before meals. It should be noted at this point that preliminary investigation in fasting diabetics showed that the action of the drug was more consistent 3 to 4 hours after it had been administered. Owing to the short time available to complete this work it was decided to stop treatment after

TABLE 1
SCHEDULE OF TREATMENT WITH CHLORPROPAMIDE

Day of treatment	Milligrams		
	Breakfast	Lunch	Dinner
First.....	250	250	250
Second.....	250	125	250
Third.....	250	125	125
Fourth and beyond.....	125	125	125

8 to 10 days in cases with poor response. We feel now that those patients who respond satisfactorily will do so after 5 to 7 days of treatment.

Analysis of Results

The patients were grouped arbitrarily according to their responses, as follows (TABLE 2):

Group A is composed of patients with excellent responses (no insulin needed), and includes those in good clinical condition who showed no glycosuria and had blood sugar values between normal limits (fasting and after meals).

Group B includes patients with good responses (no insulin needed), in whom fasting blood sugar is not more than 150 mg. per cent and not more than 200 mg. per cent 2 hours after each meal. Slight glycosuria will exist, but no acetonuria.

Group C is made up of those showing a fair response (with or without insulin), and a fasting blood sugar between 150 and 200 mg. per cent. Blood sugar after meals is between 200 and 250 mg. per cent with moderate glycosuria.

Group D includes patients showing a poor response (with or without insulin), and a fasting blood sugar of more than 200 mg. per cent and more than 250 mg. per cent after meals. Permanent and accentuated glycosuria is present, with or without acetone in the urine.

TABLE 2
VENOUS BLOOD SUGAR IN DIABETICS TREATED WITH CHLORPROPAMIDE

Type of response	Group average, mg. per cent*			
	Fasting	2 hours after breakfast	2 hours after lunch	2 hours after dinner
Excellent (A).....	115	118	136	125
Good (B).....	140	156	189	185
Fair (C).....	163	190	240	238
Poor (D).....	225	260	320	296

* Figures were obtained by blood sampling each patient at least twice at each time but on different days.

TABLE 3
RESULTS OBTAINED IN 52 DIABETIC PATIENTS TREATED WITH CHLORPROPAMIDE*

Response	No. of cases	Percentages
Excellent (A).....	10	19.2
Good (B).....	18	34.6
Fair (C).....	4	7.6
Poor (D).....	20	38.4
Satisfactory results (group A plus group B)....	28	53.8

* Age at onset of diabetes: all ages.

TABLE 4
RESULTS OBTAINED IN 30 DIABETIC PATIENTS TREATED WITH CHLORPROPAMIDE*

Response	No. of cases	Percentages
Excellent (A).....	9	30
Good (B).....	16	53.3
Fair (C).....	2	6.6
Poor (D).....	3	10
Satisfactory results (group A plus group B)....	25	83.3

* Age at onset: 40 years or more.

According to this classification (TABLE 3) we obtained satisfactory results in 53.8 per cent of the cases (groups A and B), and unsatisfactory responses in 46.0 per cent of the series (groups C and D). However, it is noteworthy that, in the group with poor response (20 cases, or 88 per cent of the group in which no satisfactory results were obtained), there were 5 patients who had developed diabetes before 10 years of age; 6 in whom the diabetes had had an onset between 11 and 20 years of age; and 6 in whom the onset age

TABLE 5
RESULTS OBTAINED IN 22 DIABETIC PATIENTS TREATED WITH CHLORPROPAMIDE*

Response	No. of cases	Percentages
Excellent (A).....	1	4.5
Good (B).....	2	9.0
Fair (C).....	2	9.0
Poor (D).....	17	77.2
Satisfactory results (group A plus group B)....	3	13.5

* Age at onset: less than 40 years.

TABLE 6
DATA ON 28 DIABETIC PATIENTS SHOWING EXCELLENT* AND GOOD† RESPONSE
TO CHLORPROPAMIDE

Case No.	Age at onset of diabetes (years)	Duration of diabetes (years)	Age (years)	Insulin U./24 hours	Body type (constitution)
1	30	3	33	0	Athletic
2	53	6	59	0	Asthenic
3	45	4	49	0	Pyknic
4	45	9	54	0	Pyknic
5	46	4	50	10	Pyknic
6	50	3	53	0	Pyknic
7	40	8	48	0	Pyknic
8	50	13	63	16	Pyknic
9	57	5	62	0	Pyknic
10	43	8	51	14	Pyknic
11	32	8	40	0	Pyknic
12	50	5	55	16	Asthenic
13	46	14	60	16	Pyknic
14	44	11	55	0	Asthenic
15	43	15	58	12	Asthenic/athletic
16	50	18	68	26	Pyknic
17	49	5	54	12	Pyknic
18	44	2	46	12	Asthenic
19	42	3	45	10	Asthenic
20	57	2	59	0	Asthenic
21	42	7	49	16	Pyknic
22	51	12	63	16	Pyknic
23	41	12	53	14	Pyknic
24	37	10	47	10	Asthenic
25	51	17	68	20	Pyknic
26	48	17	65	16	Pyknic
27	40	6	46	18	Pyknic
28	44	8	52	16	Pyknic

* Cases 1 to 10.

† Cases 11 to 28.

had been between 31 and 40 years of age. As is known from the experience gained with other orally active hypoglycemic agents that have been studied, failure of treatment is practically the rule in cases of diabetes of infantile, juvenile, and young adult types; this also seems to be the case with chlorpropamide. For example, if we take into consideration only those patients who had developed diabetes at 40 or more years of age (TABLE 4), we obtain

TABLE 7
DATA ON 24 DIABETIC PATIENTS SHOWING FAIR* AND POOR† RESPONSE TO CHLORPROPAMIDE

Case No.	Age at onset of diabetes (years)	Duration of diabetes (years)	Age (years)	Insulin U./24 hours	Body type (constitution)
1	7	3	10	40	Asthenic
2	45	1	46	10	Asthenic
3	28	3	31	30	Asthenic
4	49	1	50	0	Asthenic
5	50	7	57	24	Asthenic
6	14	2	16	36	Pyknic
7	4	1	5	12	Pyknic
8	8	6	14	54	Asthenic
9	17	12	29	52	Asthenic
10	26	8	34	54	Asthenic
11	25	10	35	50	Asthenic
12	7	5	12	46	Asthenic
13	50	4	54	8	Pyknic
14	15	17	32	52	Asthenic
15	7	6	13	58	Asthenic
16	30	6	36	30	Asthenic
17	25	4	29	46	Athletic/asthenic
18	48	13	61	64	Pyknic
19	16	4	20	64	Athletic
20	16	9	25	48	Asthenic
21	27	6	33	30	Asthenic/athletic
22	15	8	23	60	Pyknic/athletic
23	9	3	12	42	Asthenic
24	30	1	31	40	Asthenic

* Cases 1 to 4.

† Cases 5 to 24.

83.3 per cent of satisfactory results; this percentage is only 13.5 in patients with diabetes developing before 40 years of age (TABLE 5).

Examination of TABLE 6, which shows data on patients satisfactorily controlled (excellent and good results) with chlorpropamide, reveals that (1) nineteen (68.2 per cent) showed a body constitution of the pyknic type; (2) twenty-five (89.2 per cent) developed diabetes at 40 or more years of age; and (3) except for 1 patient, none received more than 20 U. of insulin every 24 hours.

On the other hand, TABLE 7, showing data on patients in whom unsatisfactory results were obtained, reveals that: (1) only 4 patients (16.6 per cent) had a body constitution of the pyknic type; (2) in 19 cases (79.1 per cent), the diabetes had developed before 40 years of age; and (3) only 4 patients (16.6 per cent) needed less than 20 U. of insulin per day for control.

Tolerance and Side Effects

Generally speaking, the drug was well tolerated at the doses used. One patient complained of epigastric distress; moderate pruritus without any apparent lesions was present in another. In a girl 10 years of age there was a slight decrease in the white blood cell count, but without concomitant neutropenia. In one instance, few red cells were found in the urinary sediment. A male patient 23 years of age, with severe diabetes, complained of somnolence, pruritus of the eyes, and moderate headache. However, we really do not know whether these symptoms were due to failure to control the diabetes during the observation period or to the use of the drug. Treatment of a young woman 31 years of age, to whom we gave chlorpropamide at a dosage of 500 mg. 3 times a day, was discontinued after 5 days because of epigastric pain. Approximately 72 hours after discontinuance the patient had a rash all over the body, together with severe itching and slight fever (100° F.). Antihistaminic drugs with small doses of prednisone improved the condition. However, 24 hours later, there was an exacerbation of the skin lesions. At this time, a closer interrogation of the patient revealed that, as self-prescribed treatment for a slight sore throat, she had taken penicillin tablets during the fourth and fifth day of the chlorpropamide treatment. Allergist Marcel Granier was of the opinion that at least this second rash could be explained by sensitization to penicillin. With proper treatment the patient recovered in a few days.

Summary

A total of 52 nonselected patients were studied from 5 to 36 days. Excellent results were obtained in 10 cases (19.2 per cent); good results in 18 patients (34.6 per cent); and fair results in 4 cases (7.6 per cent). Results obtained were poor in 20 cases (38.4 per cent); however, it should be noted that, of these 20 cases, 17 were patients under 40 years of age, 11 of whom had developed diabetes when they were less than 20; the other 6 had developed it in their 20s. As is well known, it is precisely in this type of diabetic that the oral hypoglycemic drugs have been less effective. It seems that, in a high percentage of diabetics who respond well to chlorpropamide, it is possible to maintain control with a dosage much smaller than that required for similar drugs. Many of the patients studied could be kept under control with only 125 mg. administered after breakfast, lunch, and dinner—a total dosage of 375 mg. During treatment, 1 of the patients showed a few red blood cells in the urinary sediment; another complained of a slight pruritus without an apparent lesion; and Case 12 showed somnolence, ocular pruritus, and a slight headache. It is possible that these untoward effects were caused by a diabetic unbalance rather than by the direct action of the drug. One case

showed an intense dermatitis about 72 hours after having had to discontinue chlorpropamide because of epigastric distress; nevertheless, it should be noted that this patient ingested 3 tablets daily of oral penicillin during the fourth and fifth days of treatment with chlorpropamide.

Acknowledgments

We thank Rafael Nicanor Silva and Nidia M. Fernandez for their help in the preparation of the manuscript, and Ramon Cuevas for his technical assistance.

CONSIDERATIONS FOR CONTROL NORMS TO EVALUATE RESULTS OF TREATMENT IN DIABETES MELLITUS

Miguel Ruiz-Guía

Research Department, Monte Carmelo Medical Clinic, Caracas, Venezuela

If a real picture of the diabetic problem is to be obtained, it must be considered, not only in terms of the present and in those of the individual, but also, because of the increasing incidence of this disease, its longer duration, and its hereditary characteristics, in terms of the future and of mankind.

Before 1921, the average life expectancy of a diabetic was only 4.5 years after the disease had been discovered. Today, thanks to the discovery of the hormone insulin by F. G. Banting and C. H. Best and to many advances in different fields of medicine, it is possible to say that if a diabetic is well controlled, his life expectancy will generally be the same as that of a non-diabetic of the same age and sex, and that his illness should not be an obstacle to enjoyment of a useful life. However, the question emerges: When can we say that a diabetic is well controlled? We do not intend to discuss this point at length; however, it is frequently said that, if a diabetic has no sugar in the urine or shows only traces of glycosuria and if his blood sugar remains near normal ranges, he can be considered well controlled. This could be true in many cases, but we frequently see patients whose diabetes could be considered well controlled according to these standards yet who suffer from different degenerative conditions that we think are of diabetic origin.

In other words, we think that hyperglycemia or glycosuria, or both, are indexes of the diabetes, but cannot be taken as measures of the severity of the disease. In fact, we often see patients with diabetes of long duration who have had no proper medical care for years, who show high values of sugar in the blood and in the urine, and still are free from the so-called diabetic complications. At the same time, unfortunately, we see patients who adhere faithfully to the doctor's instructions, show no glycosuria, have moderate blood sugar levels most of the time, but in whom degenerative conditions of the kidney and retinal vessels can be detected. What is more important in many of these cases is that the diabetes or, at least, the hyperglycemia has not been present for a long time. We say this because we feel that diabetes represents a very complex metabolic disturbance of which glycosuria or hyperglycemia, or both, are manifestations—the most frequent, perhaps, but not always the most important.

With the discovery of orally active hypoglycemic agents, it seems to us, the problem has become even more complex.

One thing we already know: in many diabetics blood sugar levels can be maintained, between satisfactory ranges with the use of the above-mentioned drugs.

However, another question arises: Can these patients be considered well controlled simply because their blood sugar is normal or almost so? It is

our belief that we must wait five to ten years before we can describe the condition of these patients accurately.

The situation that confronts physicians and patients in 1958 is quite different from that when insulin was first made available; insulin is a biological product and, at that time, it was unique as a diabetic specific. In other words, from now on, the results obtained with any drug in cases of diabetes mellitus should be analyzed from the following points of view: Does it satisfactorily lower the blood sugar? Does it have a better value than insulin in the prevention of the degenerative conditions? Does it have any undesirable effects? Can it be given safely for years to diabetics, who often have fragile kidneys and liver?

Briefly, we feel that, in order to obtain better data to judge the results obtained in the treatment of diabetes, it will be worthwhile to determine the type of control (such as laboratory tests, X-ray films, and electrocardiograms) that should be used and the frequency with which it should be employed. This would enable us to detect diabetic complications as early as these medical techniques are capable of uncovering them; it would be the only way to determine when to readjust our therapeutic approach without the need of waiting for the appearance of full-fledged clinical pictures such as those we see in cases of obstructive arteriopathy, intercapillary glomerulosclerosis, and advanced neuropathy. It is our feeling that we may not have been quite correct in our treatment of some of our patients, simply because our main criterion has been that of attaining satisfactory blood sugar levels.

The problem is so complex and of such great significance that possibly it would justify the establishment of an international committee to coordinate the work of researchers in this field in different countries.

Summary

In view of the current nature of the problem of diabetes and its treatment, the evolution of this condition, and the complications attendant on it (particularly those of a degenerative type), I consider it important to establish general norms for such aspects as medical controls and collateral studies. These would permit not only judgment of the effectiveness of the treatment with reference to hyperglycemia or glycosuria, or both, but would also provide an opportunity to gather data on such factors as incidence, degree of seriousness, and evolution of the so-called complications of diabetes in relation to the various types of treatment. At the same time, in order to evaluate the action of new drugs administrable *per os* and capable of lowering the glucose levels (but not necessarily controlling other diabetic manifestations), it will be necessary to plan special studies to determine their control of hyperglycemia or glycosuria and, also, comparative studies of patients treated for years with the new drugs and of those patients treated with insulin. It will also be necessary to establish the type and frequency of tests for the purpose of eliminating collateral or untoward reactions (or those of any other nature) that the hyperglycemic drugs active by the oral route are capable of producing.

THE CLINICAL RESULTS OF THE TREATMENT OF DIABETES MELLITUS WITH CHLORPROPAMIDE

Ignácio de Salcedo and Filomeno Borges

Department of Endocrinology and the Research Center of Pathological Anatomy and General Pathology of the Instituto de Alta Cultura, Faculty of Medicine, University of Oporto, Oporto, Portugal

During the last few years, numerous studies have been made to find an oral therapeutic to substitute for insulin in the treatment of diabetes mellitus, especially since the basic experiments of Loubatières clearly showed the hypoglycemic activity of certain sulfonamide derivatives.

Among the various compounds studied, carbutamide and tolbutamide soon proved of particular interest, and led to extensive and intensive experimental and clinical research, the results of which are not yet definitely established. However, it is evident that the relatively low toxicity of the compounds, especially of tolbutamide, as well as their usefulness for a specific group of diabetics can be taken for granted. In a recent report,¹ we summarized the results obtained with carbutamide and tolbutamide in the treatment of a group of 26 diabetics over a period of 21 months; in general, the results confirmed those of other investigators.

In the present report, the preliminary character of which we should emphasize immediately, we present the results obtained in the treatment of 35 diabetics with a new hypoglycemic drug, 1-propyl-3-(*p*-chlorobenzenesulfonyl)urea (P 607 or chlorpropamide), since January 1958.

Only 29 of the 35 patients have been treated for 4 months or more; we therefore base our conclusions on these cases only. Although we think that the treatment of the remaining patients can be considered a success, data on them will not be included in this report as proof or justification of the conclusions.

Of the 29 diabetics treated, 14 are female and 15 male, 35 to 68 years old, with an average age of 55.1 years.

In the female patients, 49 to 66 years old, with an average age of 57.5 years, the diabetes had an average of 6.7 years' evolution when the treatment with chlorpropamide was started. Two patients had a recent diagnosis (less than 6 months before preparation of this paper) and 2 had diabetes of 18 years' duration.

In the males, 35 to 68 years old, with an average age of 52.8 years, the average duration of the disease was equally advanced (6.1 years), with one case of 23 years and 4 of less than 6 months (FIGURE 1).

Of the 29 diabetics, 14 had been treated with tolbutamide for 12 months or longer at doses between 500 and 2000 mg. daily; 10 used between 10 and 80 U. of insulin daily (average, 43 U.); and only the 5 remaining cases were maintained on diet alone.

In all cases the treatment with the new drug began abruptly, after preliminary clinical examination and analytical tests. Except for Case 9, on

two occasions, the treatment was ambulatory and without change in the daily routine or in the diet previously followed.

Among the patients previously treated with insulin were two (cases 4 and 6) who were positively refractory to tolbutamide, as we found in an earlier trial.

Regarding the others, one female patient with a serious diabetic retinopathy began the treatment in a state of acidosis; another had an acute anthrax infection; another had a carcinoma of the upper lip that was operated after the first week of treatment; and two had tuberculosis of the lung (one cavitary).

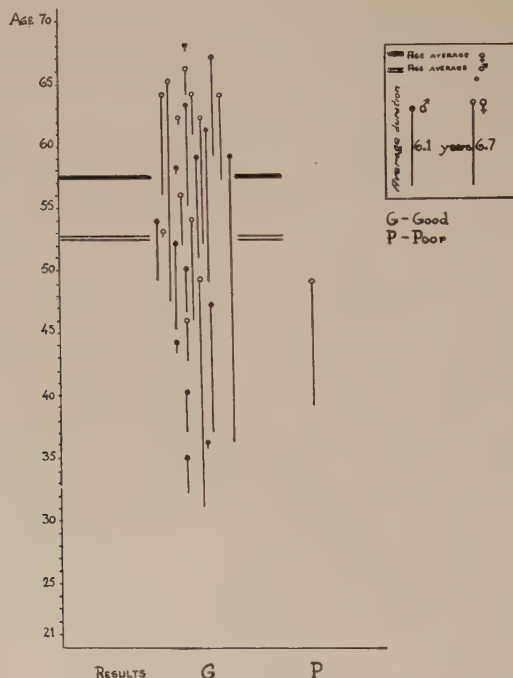


FIGURE 1. Relation of age of patient and duration of diabetes to response to treatment.

One case of cortico-adrenal hyperplasia with metabolic hyperfunction of Cushing's type, and another with a pituitary chromophobe adenoma should be mentioned. One patient developed dry gangrene of one foot during the sixth month of treatment but, in spite of this severe complication, we did not stop the medication. Thus far he has had good results with regard to blood glucose levels.

As coexistent illnesses, which may be considered minor as far as their relation to diabetes is concerned, essential hypertension was present in four patients and myocardial fibrosis in one.

With the exception of two patients, all belonged to the pyknic or pyknic-athletic groups.

In addition to a complete clinical examination, the following analytical tests were made before the beginning of the trial.

Glucose tolerance curve. Using an oral dose of 25 gm. glucose, analyses of blood glucose levels were made at 0-, 30-, 60-, and 120-min. intervals. The curve was repeated 48 hours later, with an oral dose of 1.75 gm. glucose per kilogram of body weight. The blood glucose determinations were made by the Folin-Kingsley-Reynhold method until July and, since then, by the Kingsley-Reynhold-Fister method.

NPV determination. This was determined by the hypobromite method.

Hanger's, Takata-Ara's and MacLagan's tests. In the majority of the patients, and since June, these tests have been performed by paper electrophoretic partition of serum proteins.

Blood tests. Hemoglobin determination, hematocrit, red and white cell counts, and platelet count were carried out.

After beginning treatment, repeated blood glucose determinations were made in order to maintain close watch over the effect of the medication. The other examinations were also repeated more or less frequently (an average of twice per month) in order to detect any possible systemic repercussions which might have developed. On the basis of the described technique, we think that the conclusions reached can be accepted with reasonable confidence.

In the aforementioned experiments, we endeavored to use the smallest initial dose possible. Although we administered the drug in divided doses until the middle of February, since then we have administered one dose daily, at breakfast time.

The maximum daily dose was 2 gm. (4 tablets), and was used by only 3 patients; the minimum dose in the initial treatment of 4 patients was 250 mg. In 9 cases we gave 500 mg.; the average initial dose was 922 mg. With high doses we observed no toxic reactions except dizziness and vertigo in 4 cases; these reactions were coincident with hypoglycemia.

In general, these doses—particularly those of 1 gm. or more—were administered for short periods, usually not longer than 3 days. The single exception was Case 2, a patient with acidosis to whom we gave this dosage for 3 months.

The maintenance dosage, usually reached at the end of the first week, did not exceed 500 mg. (4 cases); 12 patients were perfectly controlled on 125 mg. daily; and for 3, a daily dose of 62 mg. was sufficient. The average maintenance dosage was 248 mg.

In regard to food, all patients followed a liberal diet of carbohydrates, with quantities ranging between 300 and 400 gm. per day. The other elements were relatively balanced. We took special care to recommend to the patients that they should not vary the qualitative or quantitative substance of their diet from what they had been following before the beginning of these studies.

In view of this we think that we can present the following conclusions with reasonable assurance. However, because of the short time and the limited number of patients, the results should be considered as preliminary.

Conclusions

(1) Chlorpropamide proved active in patients who previously benefited from other types of sulfonylureas (carbutamide and tolbutamide).

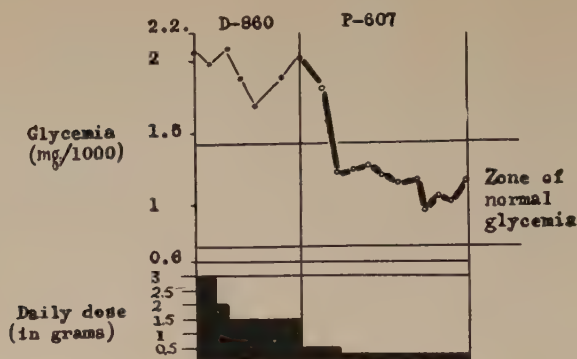


FIGURE 2

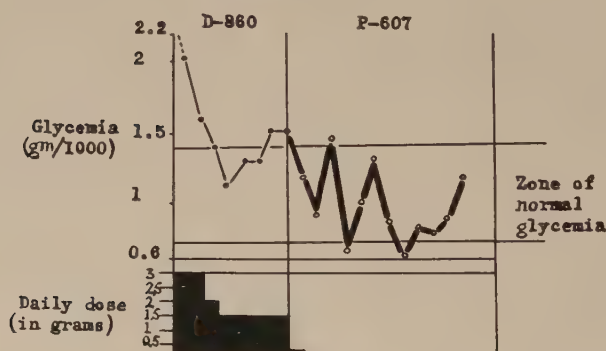


FIGURE 3

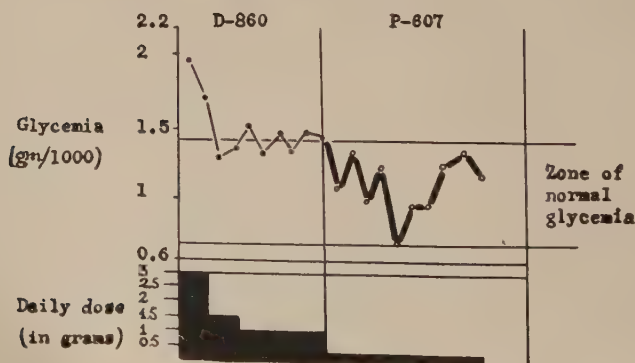


FIGURE 4

(2) Chlorpropamide was effective in two cases which had previously proved refractory to carbutamide and tolbutamide.

(3) The clinical and metabolic regulation of diabetes with chlorpropamide is more simple and more complete than with other drugs tested so far (FIGURES 2, 3, and 4). In the present group of patients, some noteworthy differ-

ences have been verified regarding the interrelation of the results obtained and the ages of the patients and, especially, the time of evolution of the disease: 37 per cent of the patients with good or excellent results are between the ages of 61 and 68 (11 cases), with an average of 64. Greater differences occur in the relation with the duration of diabetes, with 41 per cent of the patients having had the disease for 8 years or longer, giving an average duration of 11.7 years (FIGURES 1 and 5). In our previous work with tolbutamide there were no good or fair results in the patients with diabetes of more than 8 years' duration.

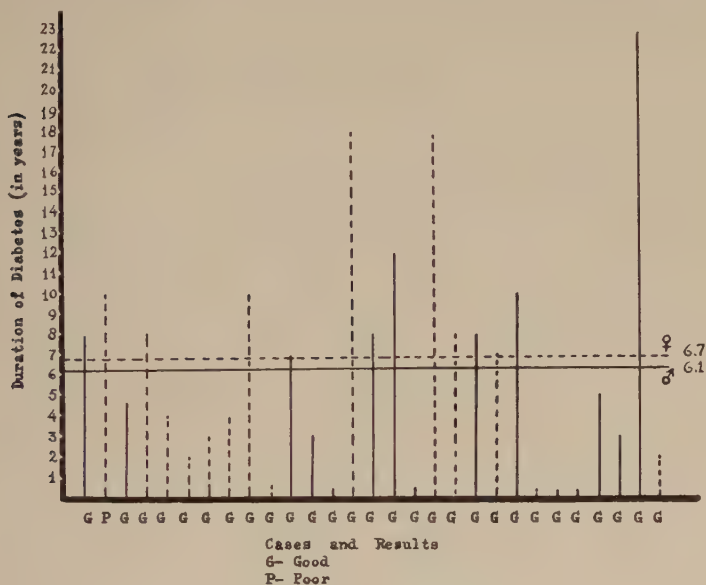


FIGURE 5. Duration of diabetes and response to treatment.

(4) In addition to six cases where the drug showed no effect and that were not included in the present report, it was found that chlorpropamide did not control the diabetes of a patient 49 years old, whose disease had begun at 39 and whose treatment started when he was in a state of marked acidosis.

This patient, the only thin diabetic of the group, had labile diabetes, requiring a daily minimum of 60 U. of insulin. In spite of the fact that the patient had severe diabetic retinopathy, we nevertheless maintained the treatment for $3\frac{1}{2}$ months, with doses varying between 1500 and 500 mg.; rapid disappearance of ketonuria and the establishment of blood glucose at lower (although still abnormally high) levels than at the beginning were verified. From a systemic point of view there were no alterations worth recording, except perhaps an improvement in visual acuity, normalization of the serum proteins, and small changes in the leukocyte count.

(5) The coexistence of a severe infection, anthrax, or of latent or active tuberculosis (in the latter case, bilateral and cavitary) does not adversely affect the efficient and rapid therapeutic action of chlorpropamide, although

it should be noted that the doses used were slightly higher than those used for the other patients in general. In one patient who developed dry gangrene of one foot, the drug nevertheless continued to be useful.

(6) On the average, the daily dose of chlorpropamide necessary for obtaining normal blood glucose levels is one quarter of the dose necessary in using carbutamide or tolbutamide, or even less.

(7) From the point of view of subjective tolerance, we must report that four patients suffered dizziness or vertigo coincident with hypoglycemia.

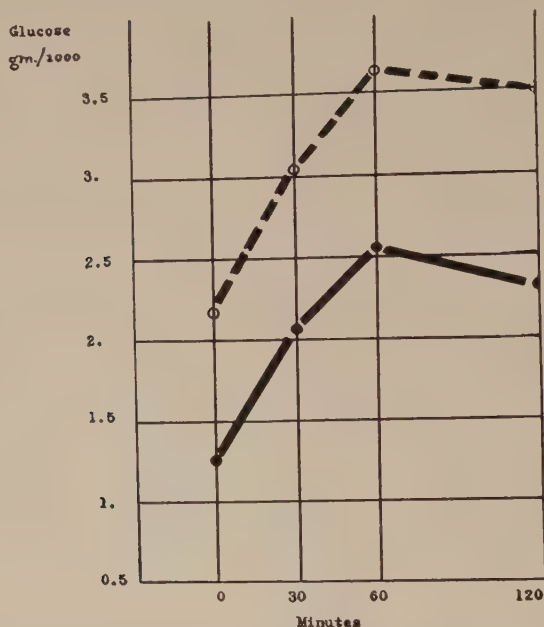


FIGURE 6. Glucose tolerance curve. Broken line represents tolerance before therapy with chlorpropamide; solid line, after three months' therapy with chlorpropamide. Curves are averages of three cases previously treated with diet alone.

In one case, a diabetic doctor had a severe melancholic depression, with suicidal ideas that promptly disappeared after the carbohydrate content of the diet was increased. No other neurological, digestive, or dermatological changes were observed in any of the patients of this group. In one case not included in the present study, the administration of chlorpropamide caused the appearance of a nettle rash in a patient who previously had shown sensitivity to tolbutamide and the sulfa drugs in general.

Seven patients reported a sudden cephalic congestion after meals, with extrasystoles in two cases and mild lung edema in one—symptoms that disappeared promptly after stopping the intake of alcohol. One of those who later resumed drinking wine did not have the same symptom. We think the phenomenon may be due to a selective inhibition of acetaldehyde dehydrogenase.

(8) Analytical observations showed that the blood glucose level remained generally normalized within the first week of treatment. In some cases, where the patient spontaneously or upon our order did not adhere to the diet, the blood glucose increased rapidly, becoming normal again without alteration of the dosage of the medicament when the former diet was resumed. With tolbutamide it was necessary to increase the dose in order to obtain the previous blood glucose level.

An average of two urea determinations per month was made, and it was found that chlorpropamide did not increase the amount of nonprotein nitro-

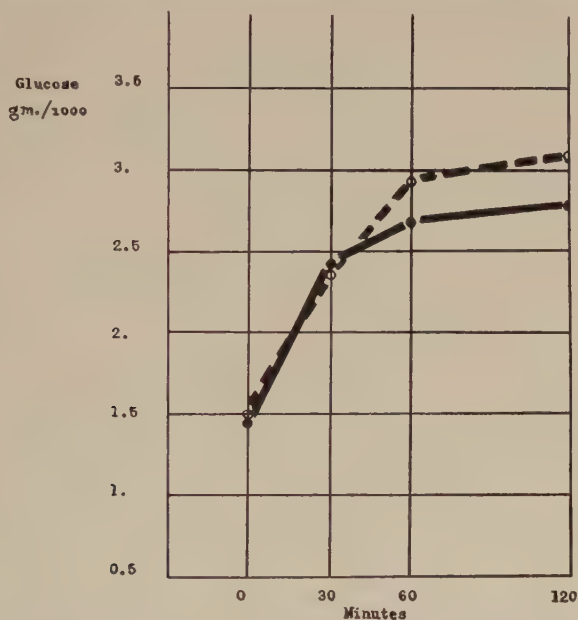


FIGURE 7. Glucose tolerance curve. Broken line represents tolerance before therapy with chlorpropamide; solid line, after three months' therapy with chlorpropamide. Curves are averages of four cases previously treated with insulin.

gen (NPN). On the contrary, the NPN normalized itself or remained unchanged, if previously high; it was found, too, that a value as high as 76 does not per se mean a contraindication.

Takata-Ara's, Hanger's, and MacLagan's tests did not show undesirable modifications, except in Case 3. In this patient, we observed high values for MacLagan's test on two occasions, but these normalized in spite of continuing the treatment. For that reason, we do not think that the drug may prove to be responsible for this abnormal finding.

Serum protein electrophoresis was performed in twenty-one cases, with normal results in all but one (Case 3).

No harmful repercussions were observed in the blood picture, which was generally tested with the same frequency as blood urea. On the contrary, we saw the normalization of the blood picture in the cases that previously had

shown alterations (6 cases). It is worth mentioning that 1 patient, who started treatment with a severe leukopenia, showed a progressive increase of the white cells from 2300 to 4900 during the trial. The hematocrit value decreased in 6 patients.

If glycosuria was present, this disappeared with the normalization of the blood sugar. The same applies to albumin in two cases and to ketone bodies in two others. In one patient traces of albumin persisted. No pathological

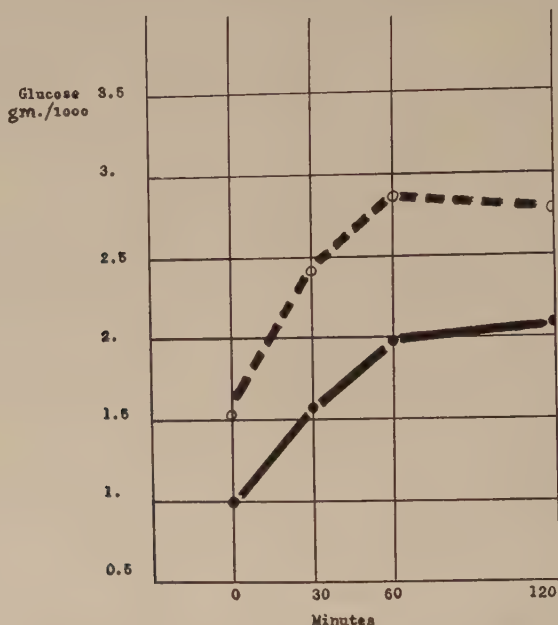


FIGURE 8. Glucose tolerance curve. Broken line represents tolerance before therapy with chlorpropamide; solid line, after three months' therapy with chlorpropamide. Curves are averages of seven cases previously treated with tolbutamide.

changes in the sediment, especially crystalluria, and no alterations in the pH appeared.

Glucose tolerance was investigated by means of an oral dose of glucose (1.75 gm./kg.) and it was found that the 2 curves, obtained at intervals of 30 days or longer, can be considered (in their conjuncture) parallel (FIGURES 6, 7, 8, and 9). This shows that there is no modification in carbohydrate tolerance.

It is our opinion that this new drug represents a major step in the treatment of diabetes mellitus.² We know that more time, more patients, and above all more experience—clinical as well as analytical—are necessary in order to obtain conclusive knowledge about this therapy. However, keeping in mind these limitations in comparison with the other drugs with which we have clinical experience (tolbutamide and carbutamide), we can say that chlorpropamide is more active and less toxic in the therapeutic dosage we have used.

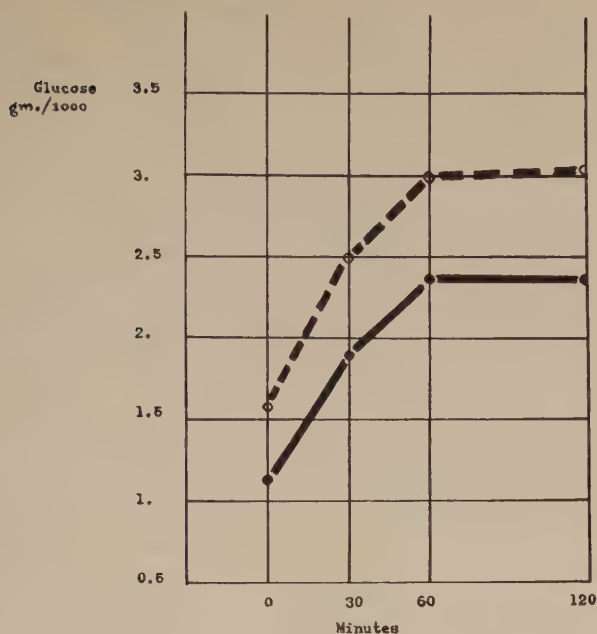


FIGURE 9. Glucose tolerance curve. Broken line represents tolerance before therapy with chlorpropamide; solid line, after three months' therapy with chlorpropamide. Curves are averages of fourteen cases.

It is worth mentioning that all the patients were maintained on almost a free diet. During this clinical trial we did not observe significant variations in the maintenance dosage.

Acknowledgment

The authors wish to thank Ernesto Morais, who permitted his laboratory to be used for the analyses mentioned in this report. We also thank Pfizer International, Inc., New York, N. Y., M. Usano of that firm, and Neo-Farmacêutica, Lda., Lisbon, Portugal, for having placed at our disposal the quantities of chlorpropamide necessary for accomplishment of the study.

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TOLERANCE FOR ALCOHOL IN PATIENTS ON CHLORPROPAMIDE

Saverio Signorelli

Istituto di Patologia Medica e Metodologia Clinica, University of Catania, Catania, Italy

By July 1, 1958 we had evaluated chlorpropamide clinically in 66 patients and had studied various sulfonylurea derivatives in approximately 600 patients with diabetes mellitus. By August 30, we had increased the number to 624 patients who had been treated for more than 1 month; of these, 88 had received chlorpropamide. Evaluating our results in this larger patient group, we can confirm the following five points relating to the control of diabetes mellitus with chlorpropamide.

(1) The hypoglycemic activity of chlorpropamide, particularly with maximum therapeutic dosages, is in some instances superior to that achieved with carbutamide or tolbutamide.

(2) The therapeutically effective dosage of chlorpropamide ranges from a maximum of 2 gm., which we used in some patients as an initial dose, to a minimum daily maintenance dose of 0.125 gm. per day; these dosages were established on the basis of satisfactory daily control of blood sugar levels and the disappearance of sugar from the urine.

(3) We observed a favorable response in 72 of 88 patients (80 per cent) and an inadequate response in 16 patients (20 per cent). It is important to note that 15 patients were less than 40 years old at the time of initiation of therapy, and that 12 of these were benefited by chlorpropamide therapy. Only three showed an inadequate response.

(4) With the initiation of therapy, and at intervals thereafter, patients were given various laboratory tests including liver function tests, blood counts, measurement of azotemia, cholesterolemia, serum proteins, and serum transaminase. From the results obtained by these laboratory tests, we observed no evidence that therapeutically effective dosages of chlorpropamide exhibited any type or degree of toxic effects.

(5) Two types of side effects necessitated the discontinuance of therapy in some patients, all of whom belonged to the group that had not shown an adequate response to chlorpropamide. The side effects were manifested as episodes of sensitivity and individual intolerance similar to those observed in response to other sulfonylurea derivatives and as episodes of specific intolerance affecting the gastrointestinal tract (nausea, sensation of abdominal fullness, constipation).

Initially, our attention was drawn to the unusual side effect associated with the ingestion of alcohol during chlorpropamide therapy by the fact that eight of twenty-three patients who were under treatment complained that after meals they experienced one or more of a number of disagreeable symptoms that consisted of sensations of warmth, flushing, nausea, giddiness and, in some, tachycardia. The patients had already come to the conclusion that

these aberrant symptoms were probably caused by the chlorpropamide, which had recently been prescribed for their diabetic condition, since the unusual sensations appeared about three or four days following initiation of therapy.

The fact that these symptoms did not appear in all patients who were receiving chlorpropamide led us to the conclusion that this side effect was not due to the therapeutic action of the drug and therefore could not be considered a side effect that could be expected to occur as a direct consequence of its administration. We reasoned, therefore, that it must be due to some set of circumstances peculiar to the patients who had developed the

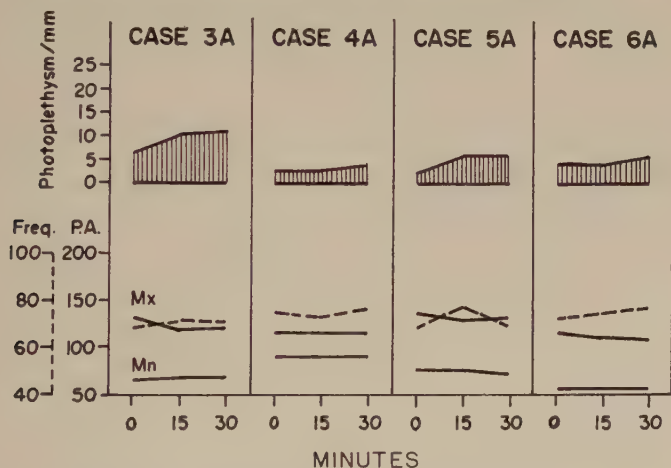


FIGURE 1. Alcohol test with normal subjects. Symbols: P.A., arterial blood pressure; Freq., heart rate (broken line); Mx, maximum; and Mn, minimum.

symptoms. We began to investigate other factors and discovered that the phenomena appeared exclusively in those patients who were accustomed to drinking wine regularly during their meals. We requested the patients to discontinue the ingestion of wine and their symptoms promptly disappeared. When they resumed wine with their meals, the symptoms returned.

While we have had other sulfonylureas under study for some time, as our total patient group shows, we have never before observed such a striking reaction to the ingestion of alcohol during treatment with a sulfonylurea. It was necessary to discontinue chlorpropamide therapy in only one patient because of the development of this phenomenon. Of course, had this patient been satisfied to discontinue the ingestion of alcohol during chlorpropamide therapy, his symptoms would have disappeared and we should have been able to continue him under chlorpropamide treatment. Such was not the case, however, and we substituted carbutamide for chlorpropamide three days after the initiation of chlorpropamide therapy. On carbutamide, this patient experienced alcohol-intolerance symptoms similar to those he had experienced while on chlorpropamide, but they were mild in nature and disappeared after a few days on continued therapy. Such a result corresponds to

the findings that some other investigators have reported concerning secondary effects observed in relation to the ingestion of alcohol by patients who were being treated with carbutamide or tolbutamide. Such symptoms of alcohol intolerance, however, have been infrequently observed with

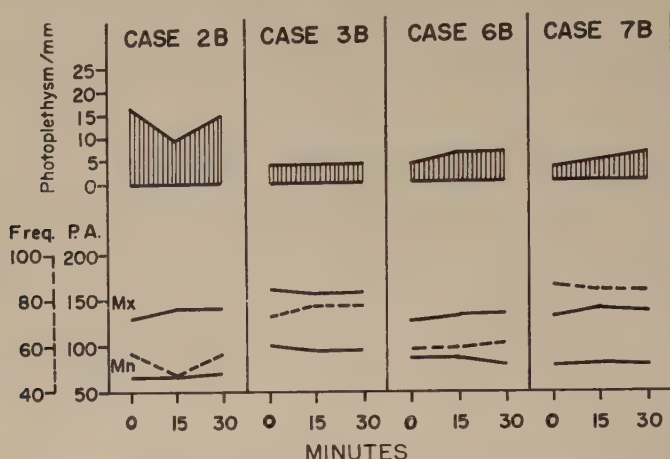


FIGURE 2. Alcohol test with diabetics not treated with chlorpropamide. Symbols as in FIGURE 1.

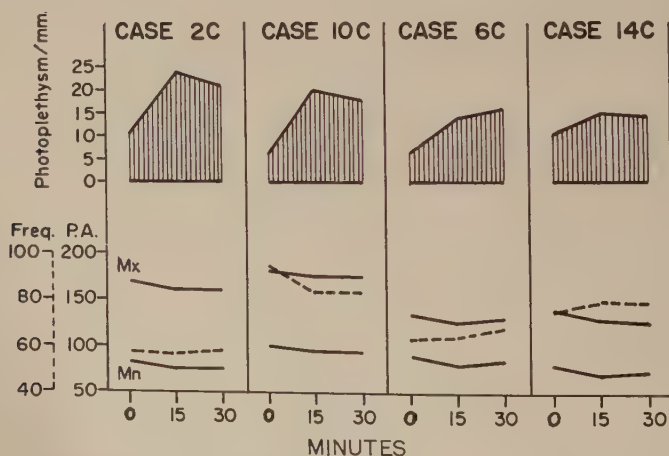


FIGURE 3. Alcohol test with diabetics under treatment with chlorpropamide. Symbols as in FIGURE 1.

these two drugs and are not considered to be of significance in their clinical application.

Following our observations of the eight patients who had experienced alcohol intolerance while under chlorpropamide therapy, we conducted the following clinical experiment.

Eight per cent (16 proof) ethyl alcohol was administered to all patients

in whom alcohol intolerance appeared. These patients had taken chlorpropamide for at least 8 days and therapy, which had successfully stabilized their diabetic state, was being continued. Within 10 to 20 min. following the ingestion of the alcohol, these patients experienced an acute sensation of warmth of the face with flushing of the same area and, in a few cases, slight giddiness similar to that caused by ingestion of a large amount of alcohol.

None of these symptoms was observed when the same dose of alcohol was given to normal subjects or to diabetics being treated with other sulfonylurea derivatives.

This intolerance reaction to alcohol has been followed in all patients, both before the ingestion of alcohol and during the appearance of the reaction, by measurement of the arterial blood pressure and by recording the heart rate, the auricular photoplethysmographic wave, and the electrocardiographic pattern.

We have not observed any variation in the electrocardiographic pattern in any patient. The values for systolic blood pressure have shown a slight tendency to reduce, usually about 10 mm. Hg, while diastolic pressure tended to reduce approximately 5 mm. Hg. The pulse rates remained within normal limits and no significant variation from normal for each individual was observed. The more relevant data recorded related to the behavior of the auricular photoplethysmographic wave. FIGURES 1, 2, and 3 show the changes that occurred in each of the patients studied.

We can state that diabetics being treated with chlorpropamide show an intolerance to alcohol more frequently than the occasional occurrence that has been observed with other sulfonylureas. However, such side effects as do occur can be completely eliminated by discontinuing the ingestion of alcohol. Thus, the patient may make a choice between ingestion of alcohol or continuing therapy with chlorpropamide.

THE TREATMENT OF DIABETES MELLITUS IN AREAS OF NUTRITIONAL DEFICIENCY

Agerico B. M. Sison, Angel A. Florentin, Herminio J. Germar
*Department of Medicine, College of Medicine, University of the Philippines,
Manila, Philippines*

The hypoglycemic effect of sulfonamides and derivatives of sulfonylureas has been observed by Janbon *et al.*¹ since 1942, but it was not until 1955 that systematic application of such findings to the treatment of diabetes mellitus was started (in France). In the same year, systematic clinical trials were undertaken in Germany by Fuchs.² Subsequently, the role of sulfonylurea derivatives in the management of diabetes mellitus has been extensively studied throughout the world.^{3, 4} While the mode of action remains to be further clarified, it is known that beneficial effects are obtained where the islets of Langerhans still possess some capacity to produce insulin.⁵⁻⁷ Hence, these drugs find particular application in the relatively mild maturity-onset type of diabetes.⁸

Since most of the diabetics seen in the Philippines are of the maturity-onset type, the introduction of these oral hypoglycemic agents met with an enthusiastic response. This was somewhat dampened by the realization that carbutamide (BZ 55) had a relatively high incidence of side reactions, while the better tolerated tolbutamide (D 860) was a less effective hypoglycemic agent.

It is the purpose of this paper to describe our clinical studies on the new sulfonylurea derivative, chlorpropamide (P 607).

Clinical Material and Method

The patients treated in the study described in this article were seen in the Diabetic Clinic of the Philippine General Hospital in Manila and in private practice. They represent part of the group used in the investigation with carbutamide or tolbutamide, or both. A careful history was obtained and physical examination made in all instances. Prior to therapy, base-line determinations included fasting blood sugar levels, urinalysis, renal function studies, hepatic function tests, and hematological and adrenocortical evaluation. Chest X-rays (for pulmonary tuberculosis) and cardiovascular studies were also done. The patients were seen in consultation by us at fortnightly intervals.

There was no uniform plan of therapy. Initial doses ranged from 500 to 2000 mg. a day, while maintenance doses ranged down to 125 mg. daily. The doses were adjusted according to the clinical and biochemical response of the patient.

The 51 patients in the series were observed for periods of from 6 to 20 weeks at the time of the preparation of this report.

Clinical Results

The response of a patient to therapy is difficult to gauge. The following arbitrary designations were adopted. The response was termed (1) excellent when the patient was rendered asymptomatic and where the fasting blood sugar level was lowered to and maintained within normal limits; (2) good when the patient was rendered asymptomatic and aglycosuric, but where fasting blood sugar was lowered to and maintained at no higher than 25 per cent above normal; (3) fair when symptoms and glycosuria were minimized but where the fasting blood sugar, although not brought within 25 per cent

TABLE 1
RESULTS OF THERAPY WITH CHLORPROPAMIDE

Response	No. of patients	Percentages
Excellent.....	14	27.46 }
Good.....	19	37.26 } 64.72
Fair.....	8	15.68
Poor.....	6	11.76 }
Discontinued treatment.....	4	7.84 } 19.60
Total.....	51	100.00

of the normal levels, could be maintained at least at 60 per cent of the initial levels; and (4) poor when no clinical or biochemical improvement could be demonstrated. In all instances, side reactions were minimal. These criteria were adopted because, in almost all instances, some degree of lowering of the blood sugar was observed, although it could not be maintained.

TABLE 1 summarizes the results obtained with chlorpropamide in the 51 subjects studied: 64.72 per cent showed excellent to good results; 15.68 per cent had a fair response; 11.76 per cent were not benefited. Four patients, or 7.84 per cent, were forced to suspend medication for various reasons.

The following cases show the pattern of an excellent response. Case 2 (FIGURE 1), F. F., 69 years, female, was recognized as a diabetic only 1 month before treatment. She had the usual symptoms of polydipsia and polyphagia. Initial fasting blood sugar was 248 mg. per cent. Treatment was started with 1500 mg. of chlorpropamide divided into 3 doses. There was prompt disappearance of glycosuria. On the fourth day of treatment she complained of slight dizziness, headache, and blurring of vision. Blood sugar was found to have dropped to 96 mg. per cent, and treatment was suspended. The disturbing symptoms promptly cleared. Returning slight glycosuria and rising fasting blood sugar to 130 mg. per cent indicated resumption of medication at a dose of 750 mg. a day. This was followed by a rapid lowering of the fasting blood sugar (FBS) to 86 mg. per cent and reappearance of the mild symptoms of dizziness and blurred vision. The dose was then adjusted downward to 500 mg. daily. The disturbing symp-

toms subsided and blood sugar was maintained at 96 mg. per cent. After about 1 month, chlorpropamide was suspended to check the need for further maintenance. There was a slight rise of FBS to 117 mg. per cent in the next 3 weeks. Resumption of the medication resulted in lowering of the blood sugar without reappearance of the hypoglycemic manifestations.

Case 7 (FIGURE 2), A. M., 54 years, male, was known to be diabetic for 21½ years and had required 30 to 40 U. of insulin for the past 2 years. An initial dose of 1000 mg. chlorpropamide resulted in a precipitous drop of

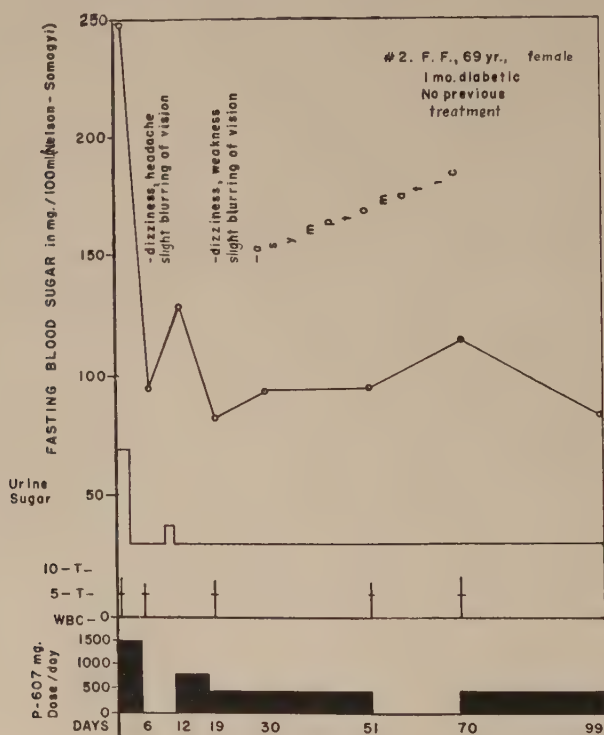


FIGURE 1

FBS from 210 mg. per cent to 90 mg. per cent in 2 days with accompanying dizziness, slight weakness, and unsteadiness of gait. These disappeared with suspension of treatment for about 5 days. Reinstitution of the drug at a dosage of 500 mg. daily produced a slow decline in the FBS from 105 to 69 mg. per cent in 31 days, without reappearance of side effects. Medication was suspended for 2 weeks and FBS rose to 85 mg. per cent. Resumption of treatment at 250 mg. daily and, later, 178 mg. daily, maintained blood sugar between 75 mg. and 90 mg. per cent.

FIGURE 3 shows an example of a good response. Case 19, D. T., 42 years, was found to have diabetes mellitus when he was confined for a pneumonitis. Initial blood sugar was 249 mg. per cent. This was controlled with NPH

insulin at dosages reduced stepwise from 25 to a maintenance of 10 U. daily. At the end of 1 month, FBS was maintained at 62 mg. per cent, and insulin was suspended. After 1 week, FBS had risen to 125 mg. per cent and carbutamide was given at levels of 500 mg. daily. This caused a drop of the FBS to 75 mg. per cent in the next 4 weeks. Carbutamide was suspended when FBS was reduced to 69 mg. per cent. The patient was next seen about 2½ months later when he observed returning glycosuria. FBS had risen to 138 mg. per cent, and carbutamide was resumed at 750 mg. daily.

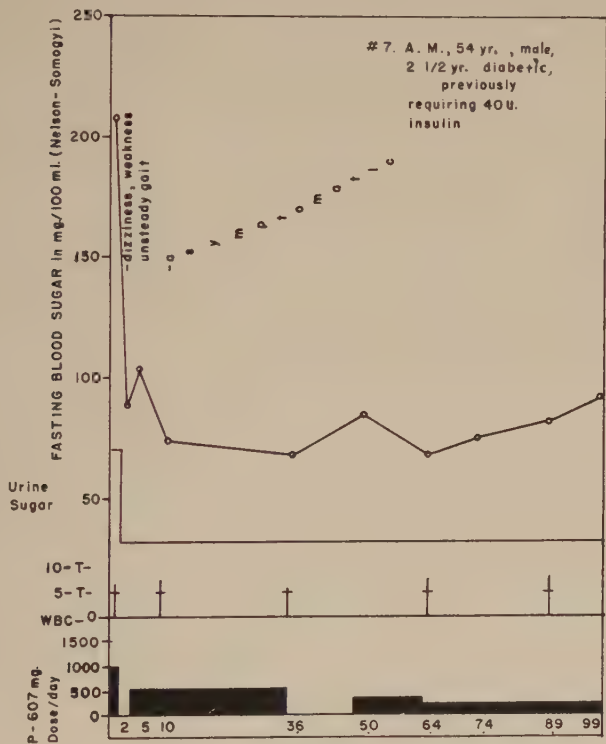


FIGURE 2

Blood sugar again showed a tendency to fall, although at a slower rate than previously. When chlorpropamide became available, the patient was shifted to it at levels of 500 mg. daily. Although there were neither symptoms nor glycosuria, the FBS showed a slow tendency to rise to 77, then 94 and, finally, 112 mg. per cent. The dose was then adjusted to 750 mg. daily. Blood sugar showed a prompt fall. At one visit, a sudden elevation of FBS to 143 mg. per cent was found to be associated with mental strain. Use of a tranquilizer, without further adjustment of diet or increase in chlorpropamide dose, caused a return to the former state of control.

FIGURE 4 depicts the course of one of the youngest diabetics in our series. Case 20, O. J., was first diagnosed at the age of 23 years. He is a male and

had pulmonary tuberculosis concomitantly. He did not receive any systematic form of treatment during the first 7 years of his diabetic life, and initial treatment consisted of 2000 mg. carbutamide daily. In 2 weeks FBS dropped from 260 mg. per cent to 143 mg. per cent, and glycosuria declined from heavy to moderate. Carbutamide was reduced to 1500 mg. daily because of the appearance of itching skin rashes. About 2½ months later, medication was suspended because of the reappearance of the cutaneous

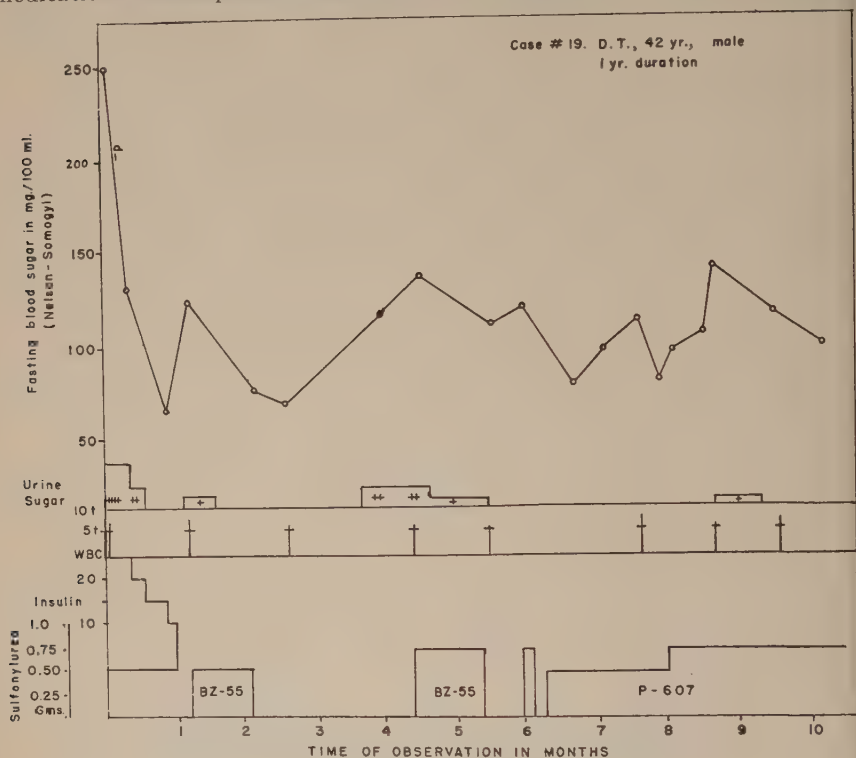


FIGURE 3

rash and a tendency to lowering of the leukocyte count. During this period, blood sugar remained about 150 mg. per cent. After 1 month on anti-histamines, the skin condition had cleared and the white blood cell count (WBC) was normal; carbutamide was resumed. Failure to lower the FBS below 150 mg. per cent, together with appearance of epigastric pain and diarrhea, caused suspension of carbutamide after 2 months. NPH insulin was given at levels of 15 U. daily. This, too, simply maintained FBS at the same level. After 2 more months, the patient complained of the injections and requested another trial with oral hypoglycemics. Tolbutamide was then available and a trial was instituted at a dosage of 1500 mg. daily. Gastrointestinal disturbance, sore throat, and leukopenia ensued and caused immediate suspension of the drug; it was necessary to resume insulin. A

combination of 15 U. NPH insulin and 1000 mg. of carbutamide produced a progressive lowering of the blood sugar far more efficiently than could be produced by either drug alone.

The insulin-carbutamide combination was suspended when chlorpropamide became available. Started at 500 mg. daily, chlorpropamide proved insufficient, and the dose was gradually increased to 1000 mg. daily. Although the blood sugar could not be maintained at the same level as that obtainable with the insulin-carbutamide combination, the effects were definitely better

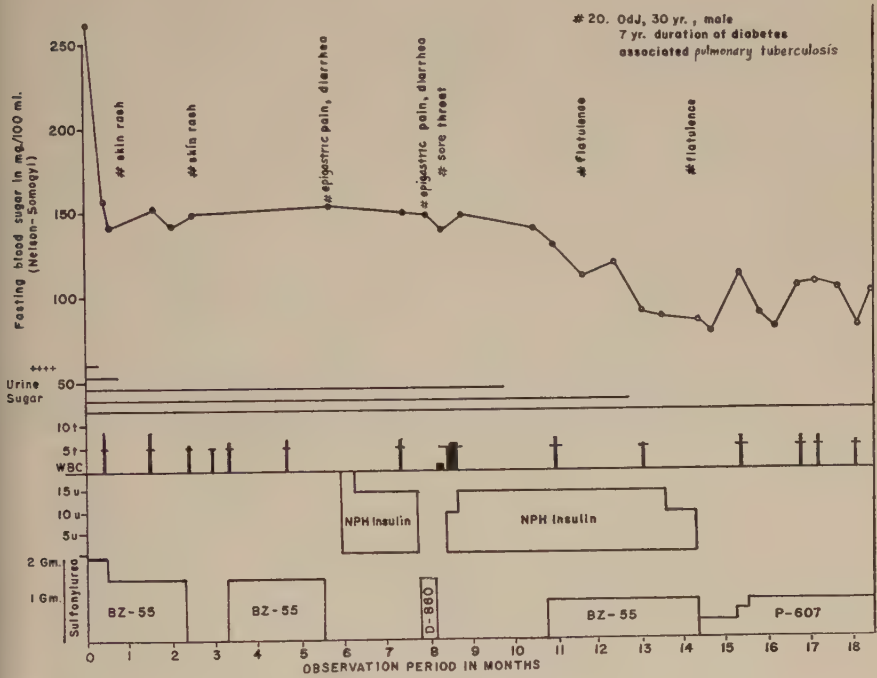


FIGURE 4

than with either 15 U. of insulin or 1500 mg. of carbutamide alone. We plan to combine a small amount of insulin with chlorpropamide.

FIGURE 5 shows an example of a poor response. Case 33, V. L., is a 51-year-old male who had diabetes for 9 years and required 40 U. of insulin for control. He showed evidence of hepatic dysfunction, particularly in the abnormal results of flocculation tests. He was started on 750 mg. of chlorpropamide daily. Because of lack of response dosage was increased to 1500 mg. daily. There was a slight drop of FBS from 225 mg. per cent to 148 mg. per cent but, without changing the dose or diet, the blood sugar could not be maintained at this level. The addition of 15 U. of insulin arrested the upward spiraling of the blood sugar level, but could not reduce it to normal levels.

FIGURE 6 represents the interesting case of a 58-year-old female who was

known to be diabetic for 2 years, and had been treated with diet and insulin. She was admitted to the hospital for a lower abdominal mass, diagnosed by the Department of Gynecology as a cystadenoma of the ovary. Surgical intervention was projected, and the patient was referred for control of the diabetic picture prior to surgery. When first seen, she had a blood sugar level of 194 mg. per cent, heavy glycosuria, and pruritus vulvae. She was placed on 2000 mg. of chlorpropamide daily. FBS dropped to 112 mg. per

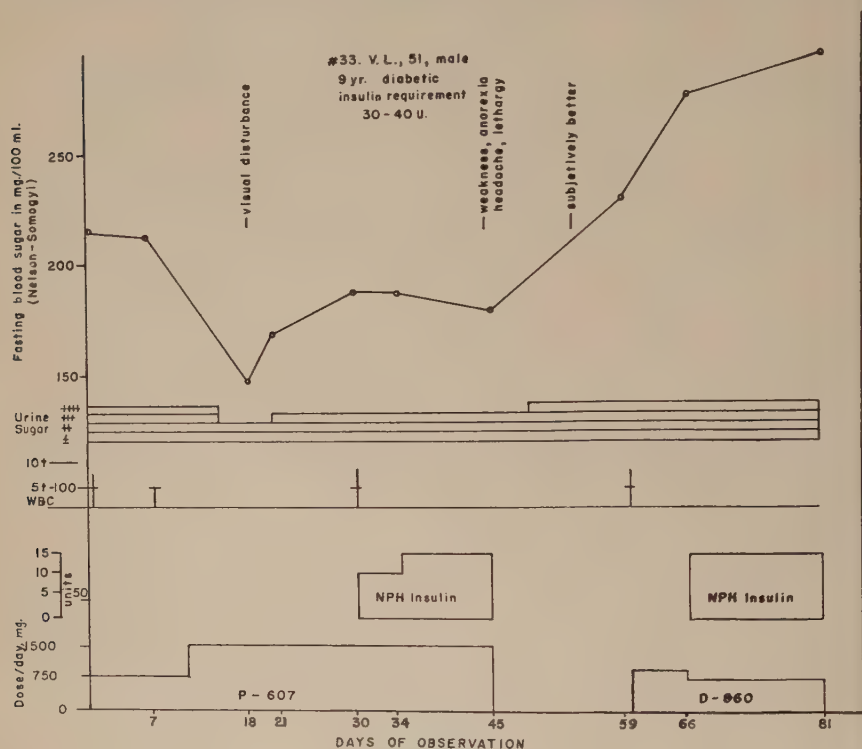


FIGURE 5

cent in 3 days and glycosuria was reduced to a minimum. A panhysterectomy was performed the next day, and the postoperative course was uneventful. The diabetic picture remained under control. Two weeks later it was necessary to reduce the medication to 1000 mg. daily because of a transient weakness and dizziness. Attempts to reduce the dose further resulted in a rise of the blood sugar; a maintenance dose of between 750 mg. and 1000 mg. daily was found adequate.

The pattern of response of the patients treated in our study was very encouraging. There is a uniform tendency to lower the initial hyperglycemia and to maintain the lowered blood sugar level. FIGURE 7 depicts the relationship between the blood sugar level in patients whose response was rated fair to excellent and the amount of chlorpropamide required to

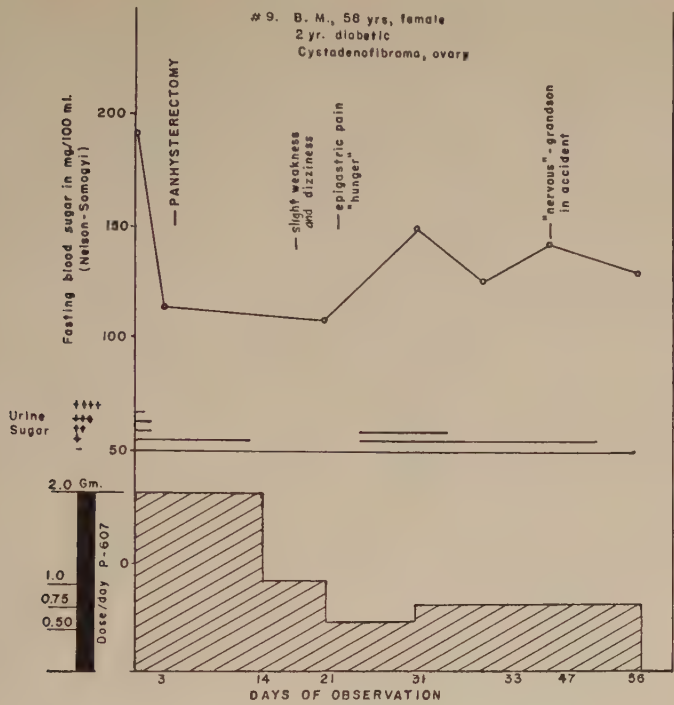


FIGURE 6

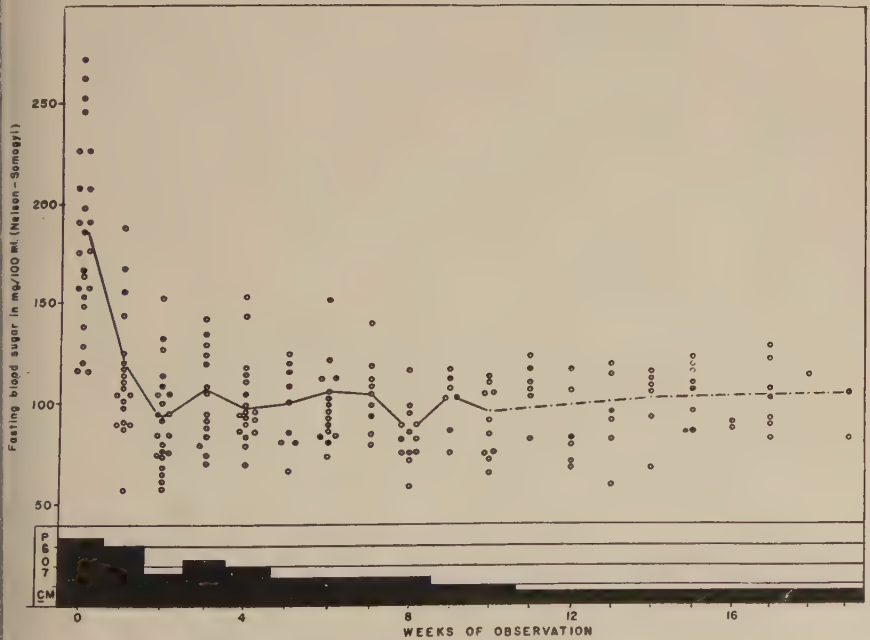


FIGURE 7

produce those results. Initial doses of slightly more than 1.5 gm. daily were sufficient to produce a reduction of the fasting blood sugar from an average of about 180 mg. per cent to about 118 mg. per cent in 1 week. The fasting blood sugar could be maintained at about 100 mg. per cent by maintenance doses as low as about 400 mg. daily. These doses are certainly very much smaller than those we utilized previously with the other sulfonyl-urea derivatives.

Side Effects

The patients were carefully questioned at each visit about any untoward effects. TABLE 2 shows the side reactions that were encountered in the

TABLE 2
SIDE REACTIONS OBSERVED WITH CHLORPROPAMIDE THERAPY*

Reactions	No. of cases
Transient weakness.....	13†
Transient dizziness and headache.....	9
Transient blurring of vision.....	8
Unsteadiness of gait.....	3
Sialorrhea.....	3
Increased "hunger pains".....	2
Flatulence.....	2
Localized dermatitis.....	1
Exfoliative dermatitis.....	1†
Generalized allergy.....	1†
Leukopenia.....	0
Purpura or anemia.....	0

* Duration of treatment of 51 patients: 4 months.

† Suspension of chlorpropamide therapy caused disappearance of symptoms.

51 patients during the period of observation. The most frequent complaints were those of transient weakness, dizziness, headache, and blurring of vision. They occurred with greatest frequency at the start of the investigation, when we used doses of chlorpropamide approximating those of tolbutamide. The complaints were often registered about 3 to 5 days after onset of treatment, and promptly cleared when medication was suspended. The effects have been attributed to a rapid reduction of the blood sugar levels as shown by close observations and repeated blood sugar checks similar to those depicted in FIGURES 1 and 2. Reduction of the initial doses of chlorpropamide to an average of 750 mg. a day, with slight adjustment of the dose according to the individual response of the patient, diminished the frequency of the complaints in subsequent patients.

Four patients did not continue treatment. In 2 patients the appearance of hypoglycemic reactions, 3 days after initial doses of 2 gm. of chlorpropamide daily, resulted in their voluntary return to insulin treatment. One patient developed generalized urticarial rashes and complained of nausea, vomiting, and difficulty of breathing after ingesting 3 tablets of the medication. The

Further developed exfoliative dermatitis during the course of treatment. This is graphically shown in FIGURE 8. Case 36, R. V., 41 years, female, was known to be diabetic for 10 years and had an insulin requirement that varied from 20 to 40 U. daily. She also had pulmonary tuberculosis and a family history of allergic tendency, although the actual allergens could not be pinpointed. Initial FBS was 190 mg. per cent, and she was started on 500 mg. chlorpropamide daily. Weekly visits thereafter showed progressive

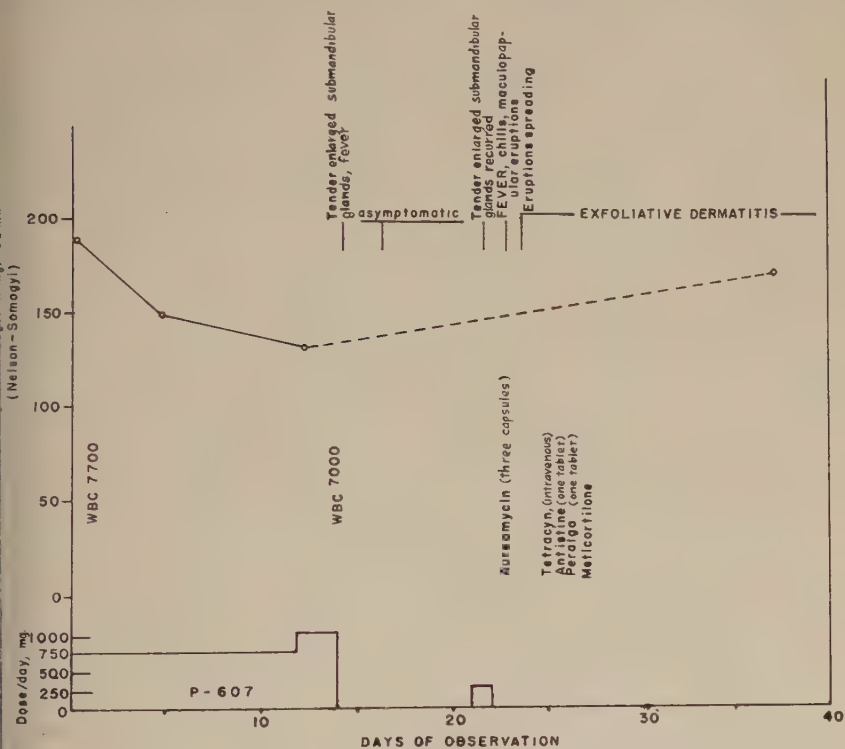


FIGURE 8

lowering of the blood sugar levels to 143 mg. per cent and then to 130 mg. per cent; she was asymptomatic. On her second visit, the dose was increased to 1000 mg. daily. After 2 days she complained of slight fever and tender, enlarged submandibular glands. Blood examination showed normal WBC. She was then advised to suspend chlorpropamide, and all symptoms subsided 2 days later.

After one week, the patient again felt tenderness and enlargement of the submandibular glands and slight fever. She took one tablet of chlorpropamide at noon and complained of heaviness of the body, higher fever, chills, and appearance of maculopapular eruptions over the chest and face in the afternoon. On advice of another physician, she took three capsules of Aureomycin. There was no relief from the fever and the skin eruptions

continued to spread. Subsequent medication with Tetracycline, Antistine, and Peralga brought no relief and her condition suddenly became a full-blown exfoliative dermatitis. When seen in the hospital, she appeared in critical stage and had to be treated with insulin and Meticortelone. These events do not with certainty indict chlorpropamide as the culprit, but the case is being presented as one where a dangerous skin disease occurred during the course of treatment. The patient recovered completely from the exfoliative dermatitis.

TABLE 3
COMPARISON OF DIABETIC DEATH RATES OF DIFFERENT COUNTRIES

Country	Year	Population	Deaths from all causes	Deaths from diabetes	
				Number	Rate per 100,000
Australia.....	1949	7,912,638	75,260	1,473	18.6
United States.....	1950	150,697,000	1,450,270	25,010	16.6
Norway.....	1949	3,233,000	29,082	440	13.6
Canada.....	1950	13,821,000	123,649	1,545	11.2
France.....	1950	41,900,000	526,280	3,763	9.0
Italy.....	1950	46,279,500	448,971	3,544	7.7
Spain.....	1949	28,023,000	316,308	1,492	5.3
Mexico.....	1945	22,233,200	433,694	811	3.6
Colombia.....	1948	10,776,900	154,392	265	2.5
Japan.....	1950	83,200,000	908,801	2,007	2.4
Philippines.....	1956	21,856,875	219,719	137	0.6

TABLE 4
INCIDENCE OF DIABETES MELLITUS AND DIABETIC ACIDOSIS IN PATIENTS AT THE PHILIPPINE GENERAL HOSPITAL, MANILA, PHILIPPINES, 1945-1955

Number of hospital admissions.....	181,116
Number of medical cases.....	32,112
Admissions and cases diagnosed as diabetes mellitus.....	639 (1.9 per cent)
Admissions and cases diagnosed as diabetic acidosis.....	10

TABLE 5
COMPARISON OF INCIDENCE OF DIABETIC ACIDOSIS IN THE PHILIPPINES AND THE UNITED STATES

	Philippine General Hospital	New England Deaconess Hospital	Philadelphia General Hospital
Period covered.....	1945-1955	1946-1951	1931-1936
Number of diabetic admissions.....	639	10,200	3,009
Number of diabetic comas.....	10	153	268

TABLE 6
COMPARISON OF DIETARY INTAKE AND DIABETIC MORTALITY RATES IN DIFFERENT COUNTRIES

Country	Total calories	Distribution of caloric intake								Diabetes mellitus mortality per 100,000	
		Cereal	Root tubers	Sugar	Fats	Legumes	Fruits and vegetables	Meat	Milk		Wine
Australia.....	3128	872	93	582	420	28	106	755	282	—	18.6
United States.....	3249	887	139	515	502	105	210	524	367	—	16.6
Norway.....	3129	1147	242	391	631	27	40	326	313	12	13.6
Canada.....	3109	943	177	518	464	81	118	442	366	—	11.2
France.....	3012	1203	247	247	320	100	67	303	222	298	9.0
Italy.....	2627	1606	63	80	256	158	52	148	108	156	7.7
Spain.....	2788	1416	180	143	360	158	86	324	101	110	5.3
Mexico.....	1909	1004	42	197	106	108	96	197	155	4	2.6
Colombia.....	1934	768	313	98	93	45	100	279	233	5	2.5
Japan.....	2268	1559	157	163	41	147	83	107	11	—	2.4
Philippines.....	2021	1115	264	124	130	79	23	218	68	—	0.6

There were no evidences of impairment of renal function or liver function, or of injury of the bone marrow in any of the cases under observation.

Discussion

Diabetes mellitus is not a major cause of death in the Philippines. TABLE 3⁸ shows a comparison of pertinent figures available from different parts of the world.⁹ Among the countries on this side of the Pacific, Australia ranks high, with diabetes mellitus accounting for 18.6 deaths per 100,000 population; Japan has only 2.4 per 100,000 population; and the Philippines had only 137 deaths ascribed to the disease in 1956, or a mortality rate of 0.6 per 100,000 population.

Figures from the Philippine General Hospital (TABLE 4) for the period 1945 to 1955 show that 1.9 per cent of the patients admitted to the Department of Medicine were diagnosed as cases of diabetes mellitus; however, of the 639 cases occurring during this period, only 10 showed diabetic acidosis. In comparison (TABLE 5), 5-year surveys in the New England Deaconess Hospital, Boston, Mass., and the Philadelphia General Hospital, Philadelphia, Pa., show a large number of patients with diabetic acidosis.⁹

These statistics support the observation by most practitioners in the Philippines that the great majority of diabetics there are mild and that juvenile or growth-onset type of diabetes is a rarity. The factors that may explain this are not entirely clear. One suggestion that may be tenable is that the Filipino diet,¹⁰ which is relatively low in fat and moderate in calories, approximates the diabetic diet. Inasmuch as dietary restriction is an accepted method of treatment, it is postulated that the somewhat nutritionally deficient diet contributes to a milder course of the disease.

TABLE 6 shows a comparison of the general food habits from different countries as gleaned from Food and Agriculture Organization (FAO) reports.¹¹ There appears to be a rough correlation between the nutritional adequacy of the diet and the mortality rate for diabetes mellitus, particularly with respect to the total calories and the fat content. The evidence, while not conclusive, points to a trend which deserves further investigation.

The use of the sulfonylurea derivatives in diabetes mellitus ushers in a new era in management of the disease. This is especially true in areas where the diabetes found is mild and where resistance and the incidence of coma are low. Such conditions obtain in the Philippines and, for this reason, we have been very much interested in studying chlorpropamide.

Summary

The great majority of cases of diabetes mellitus in the Philippines are of the maturity-onset type. The incidence of diabetic acidosis and of juvenile diabetes is small.

Chlorpropamide has shown great effectiveness in controlling diabetes mellitus.

The dose of chlorpropamide found effective in controlling the disease is much smaller than expected; initial dosages of 750 to 1000 mg. daily, and maintenance doses of 250 to 500 mg. daily were found to be adequate.

Few side effects were encountered. The most frequent were symptoms hypoglycemia produced when fairly large doses were employed.

A case of exfoliative dermatitis was reported to have developed in one patient during the course of treatment.

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THE PROBLEM OF DIABETES IN THE SINGAPORE POPULATION AND THE IMPACT OF ORAL ANTIDIABETICS ON ITS MANAGEMENT

Ho Yuen

Singapore General Hospital, Singapore

In Singapore, where the population is made up of many races or ethnic groups and where literacy is far from universal, diabetes poses problems, particularly in management, quite different from those met with in the more highly developed countries of America and Europe. Singapore has a population of 1,500,000, consisting mainly of Chinese (76.4 per cent), Malays, and Indians. It was from this population that subjects of this study were drawn, and the investigation was carried out in 1 of the 2 teaching medical units in the Singapore General Hospital.

The Size of the Problem

In 1957 the number of patients with diabetes newly registered in 1 of 2 diabetes clinics at the hospital totaled 377. These, together with patients already on the register, gave a total clinic attendance of 7,217 for the year. There is as yet no unified center for the treatment of diabetes, and such treatment is undertaken at the Singapore General Hospital, the Tuberculosis Hospital, outpatient clinics of the outpatient service, and by private practitioners. Therefore, statistical data on the numerical size of the problem posed by diabetes mellitus are difficult to obtain. However, there is little doubt that diabetes constitutes a large part of the problem of the care of the health of the population.

Etiological Factors

While definitive etiological factors in cases seen in Singapore and elsewhere may be largely similar, the emphasis on any particular etiological factor seems to vary. Thus, in Singapore, diabetes in children is decidedly uncommon, and the disease frequently manifests itself in middle life.

The maximum incidence falls within the age group of 36 to 65 years. The sex incidence also differs from that seen in other countries, as the disease is commoner among males than females. In its distribution among the various races, the disease is commoner among Indians in a population predominantly Chinese. The majority of patients comes from the low-income group in which carbohydrates, especially in the form of rice, constitute the major part of the diet.

Presenting Symptoms

Relatively few of our patients come to us because sugar was found in the urine on routine examination. Thirst and frequent passage of large quantities of urine, especially at night, comprise the symptoms most frequently complained of, while not a few come for medical advice because they observed

nts gathering about their urine or the toilet. Infective complications, chiefly carbuncle and pulmonary tuberculosis, cause them to be seen by our surgical colleagues or in the tuberculosis hospital. While a wide variety of complications is encountered, the incidence of diabetic coma is definitely low. In one of the two medical units at the General Hospital, only seven patients in diabetic coma were seen in the past year. This is not surprising, as the great majority of the diabetics have diabetes of late onset.

Problem in Management

It is generally known that the Chinese culture emphasizes good food and, in Singapore, this attitude toward food is shared by the other communities. Hence, it is extremely difficult, if not impossible, to expect our patients to adhere to a strict diabetic diet for long. It is chiefly for this reason that hospital in-patient treatment, with its dietary regimen, is unsatisfactory, as the patient's home food differs both in quality and quantity from that given him in the hospital. Consequently, the disease is treated largely on an out-patient basis, where the patient is allowed a "free" diet. Only those with infective and other severe complications are admitted to the hospital wards. In outpatient treatment, while sugar and sweetened foods are strictly prohibited, other carbohydrates (including rice) are offered at each meal. In this way our patients are able to arrive at a fairly constant carbohydrate intake frequently higher than the intake they can be allowed on dietary treatment alone.

As observed earlier, the majority of our patients come from the poorer segment of the population, and management of the disease and the initiation into a diabetic life are rendered more difficult by their illiteracy. Printed instructions are of little value. Each patient must be instructed in turn and where possible, if language difficulties can be overcome, some understanding of the disease process must be brought within his grasp. If this is achieved, the illiterate diabetic will adhere faithfully to the advice of the physician and his disease will be brought under control quickly. Until the advent of oral drug therapy the overwhelming majority of patients received insulin treatment and were carefully taught to inject themselves and to test and chart their urinary sugar results.

As soon as it became generally known that tablets were available for the treatment of diabetes, almost every patient saw in this a relief from daily injections. The demand for oral therapy was persistent and almost universal in the diabetes clinics, and carbutamide and tolbutamide, were tried and reported on by Tan Bock Yam and Ronald Wells (1957).

Clinical Trial with Chlorpropamide

In Singapore, when chlorpropamide became available for investigational use it was decided to conduct a clinical trial in an attempt to assess its efficacy in the treatment of diabetes mellitus.

Material. The patients who underwent a clinical trial with chlorpropamide comprised a group of sixty-seven cases selected at random, regardless of relation to the type or severity of the diabetes, age, sex, or dietary habits.

However, it was felt that patients with infective complications or acidosis, or both, should be excluded, as the trial was conducted on an outpatient basis so that direct supervision could not be as close as when patients were hospitalized.

The age of patients at the onset of diabetic symptoms and their sex and race distribution are shown in TABLES 1 and 2.

TABLE 1
AGE AT ONSET OF DIABETES AND SEX DISTRIBUTION OF PATIENTS ON CHLORPROPAMIDE

Age*	26-30	31-35	36-40	41-45	46-50	51-55	56-60	61-65	66-70	Total
Male.....	2	6	7	7	6	8	4	1	1	42
Female.....	0	0	5	5	9	3	1	1	1	25
Total.....	2	6	12	12	15	11	5	2	2	67

* Average age at onset of diabetes: 45.8 years.

TABLE 2
RACE DISTRIBUTION OF PATIENTS ON CHLORPROPAMIDE

	No. of patients*				
	Chinese	Indian	Malay	Others	Total
Group A.....	12 (44.4)	13 (48.1)	nil	2 (7.5)	27 (100)
Group B.....	11 (44.0)	9 (36.0)	4 (16.0)	1 (4.0)	25 (100)
Group C.....	2 (13.3)	9 (60.0)	4 (26.7)	nil	15 (100)
Total.....	25 (37.3)	31 (46.3)	8 (11.9)	3 (4.5)	67 (100)

* Figures in parentheses are percentages of total.

All patients were of the sthenic or plethoric type of bodily configuration, and all displayed symptoms referable to their diabetes.

Method of trial. The 67 trial cases were divided into three groups: Group A, those who did not have any previous treatment; Group B, those who were treated with tolbutamide; and Group C, those treated with insulin prior to chlorpropamide therapy.

All patients were examined clinically, and urinalyses, glucose tolerance tests, X-ray examinations of the chest, and blood counts were done; the first and last were repeated at intervals.

The patients attended the clinic weekly while dosage of chlorpropamide was adjusted according to the urinary sugar reaction to Benedict's reagent; the urinary tests (checked by the laboratory at intervals) were done by the

Case No.	Reference No.	Race*	Sex	Present age	Age at onset (years)	Blood sugar mg. %		Final Chlorpropamide dosage gm./day	Duration of treatment (weeks)	Quality of control	Side effects	Thrombocytes per cu. mm. blood	Remarks
						Fasting	Max.†						
1.	N1875	Ind.	M	33	32½	138	266	0.5	22	good	Furuncles	180,000	
2.	N1429	Ind.	M	50	50	246	416	0.75	14	good	giddiness initially	—	
3.	N2261	Ind.	M	44	41	288	302	0.75	21	good	epigastric discomfort	150,000	
4.	N2253	Ch.	F	53	53	235	392	1.0	17	fair	nil	130,000	random blood sugar: 152 mg. %
5.	N2467	Ind.	M	55	53	306	540	0.5	15	good	nil	225,000	random blood sugar: 61 mg. %
6.	N3140	Ch.	M	54	49	216	294	0.5	12	good	sleepiness	135,000	
7.	N3246	Ch.	F	54	54	218	310	0.75	4	poor	nil	—	
8.	N3247	Eur.	F	54	49	216	312	0.25	10	good	constipation	180,000	
9.	N3250	Ind.	M	56	54	243	400	0.5	9	good	giddiness	140,000	cataract
10.	N3269	Ch.	F	51	50	204	334	0.5	11	good	nil	80,000	
11.	N3357	Filip.	F	49	49	86	217	0.5	10	good	nil	200,000	
12.	N2227	Ch.	M	69	66	210	—	0.25	14	good	anorexia	80,000	hypertension
13.	N3440	Ch.	M	51	47	—	—	0.75	10	good	nil	80,000	palp. liver
14.	N3502	Ind.	M	35	—	90	236	0.5	8	good	nil	165,000	
15.	N3505	Ch.	F	53	53	184	280	0.5	8	good	nil	100,000	
16.	N3737	Ind.	M	49	40	172	268	0.5	6	good	nil	—	
17.	89/57	Ch.	F	51	46	212	370	1.0	2	poor	profuse sweating	—	Pt. refused to cont. with P607
18.	N3819	Ind.	M	38	37	235	444	0.5	7	fair	nil	—	
19.	N3972	Ind.	M	39	39	234	370	0.75	5	good	constipation	—	
20.	N4024	Ch.	M	53	52	—	—	1.0	5½	good	nil	—	furuncles
21.	N3443	Ind.	M	37	34	128	212	0.25	3	good	nil	—	
22.	N4001	Ch.	M	39	37½	227	392	1.0	4	good	nil	—	
23.	N3664	Ind.	M	44	42	332	412	0.5	8	good	constipation	250,000	peripheral neuritis
24.	N3499	Ind.	F	46	45	184	302	0.75	8	good	nil	130,000	
25.	N4178	Ch.	M	38	38	238	366	1.0	4	poor	nil	—	
26.	N3620	Ind.	M	38	38	—	—	0.5	8	good	nil	150,000	appendix abscess
27.	N3561	Ch.	F	40	39	—	—	0.75	8	fair	nil	60,000	cataract both eyes

* Key: Ind., Indian; Ch., Chinese; Eur., European; Filip., Filipino.

† In glucose tolerance test.

TABLE 4
CLINICAL DATA OF 25 DIABETES PATIENTS (GROUP B) PREVIOUSLY TREATED WITH TOLBUTAMIDE

Case No.	Refer-ence No.	Race*	Sex	Pres-ent age	Age at onset	Blood sugar, mg. %		Final dosage		Duration of treatment		Quality of control		Side effects		Throm- bocytes per cu. mm. blood	Remarks
						Fast- ing	Max.†	Chlor- prop- amide gm./ day	Tol- but- amide gm./ day	Chlor- prop- amide (weeks)	Tol- but- amide (months)	Chlor- prop- amide	Tol- but- amide	Chlor-prop- amide	Tol- but- amide		
28.	1878/56	Ch.	M	67	62	130	340	1.0	1.0	2	12	poor	good	weak, tired anorexia	nil	—	patient refused to continue with chlorpropamide
29.	N832	Malay	M	31	28	220	370	0.75	0.5	11	18	fair	fair	nil	nil	100,000	
30.	2385/56	Ind.	M	52	44	283	444	0.5	1.5	7	16	fair	fair	nil	nil	180,000	
31.	N1413	Cey.	M	52	47	233	396	0.25	1.5	12	9	good	good	nil	nil	120,000	
32.	N3145	Ch.	F	37	37	106	243	0.75	1.0	6	1	fair	good	erythema- tous rash	nil	280,000	
33.	462/57	Ind.	M	54	51	256	555	0.75	1.5	4	2	poor	poor	nil	nil	—	unreliable
34.	N833	Ch.	F	52	50	—	—	1.0	1.5	9	8	good	good	purpuric rash	nil	180,000	
35.	N1773	Ind.	M	46	42	142	242	0.5	1.0	12	3	good	good	erythema- tous rash	nil	60,000	
36.	1394/57	Ch.	F	55	48	—	—	0.75	1.5	5	12	fair	poor	nil	nil	—	
37.	N1187	Ind.	M	65	60	200	—	0.5	1.5	11	6	good	fair	constipation	nil	—	
38.	2387/56	Malay	M	37	35	200	284	0.75	1.5	12	15	better	good	nil	nil	200,000	
39.	N1334	Ch.	M	57	55	200	285	0.75	1.5	11	8½	good	fair	nil	nil	210,000	
40.	651/57	Ch.	F	47	46	—	—	0.5	1.5	13	11	good	good	giddy	nil	160,000	
41.	N1567	Ch.	F	74	69	120	240	0.5	1.5	2	4	poor	fair	marked giddiness	nil	—	chlorpropamide discontinued
42.	N998	Ch.	F	52	42	152	270	0.75	1.5	8	16	good	good	nil	nil	150,000	random blood sugar: 121 mg. %
43.	N655	Ind.	M	56	54	350	714	0.5	0.5	10	10	good	good	constipation	nil	90,000	chlorpropamide discontinued
44.	N597	Sikh	F	56	44	250	362	1.0	1.5	6	7	fair	good	nausea vomiting	nil	—	
45.	608/56	Ind.	M	54	51	178	392	1.0	1.5	9	12	good	good	nil	nil	180,000	
46.	N1652	Ch.	F	50	49	356	540	1.0	1.5	7	10	good	fair	erythema- tous rash	nil	110,000	
47.	705/56	Ch.	F	44	41	200	326	0.5	1.5	9	13	good	good	nil	nil	160,000	
48.	N595	Malay	F	47	45	—	—	0.25	0.5	7	6	good	good	nil	nil	136,000	random blood sugar: 133 mg. %
49.	N837	Malay	F	38	35½	224	354	1.0	1.5	6	13	good	good	constipation	nil	200,000	
50.	N503	Ind.	F	65	62	217	392	0.75	1.5	13	10	good	fair	nil	nil	—	
51.	1980/56	Ch.	F	53	49	167	350	0.75	1.5	6	18	good	fair	nil	nil	—	
52.	N645	Ind.	M	55	51	—	—	0.75	1.5	5	10	good	fair	nil	nil	—	

* Key: Ch., Chinese; Ind., Indian; Cey., Ceylonese.

TABLE 5
CLINICAL DATA OF 15 DIABETES PATIENTS (GROUP C) PREVIOUSLY TREATED WITH INSULIN

Case No.	Refer- ence No.	Race*	Sex	Pres- ent age	Age at onset	Blood sugar, mg. %		Final dosage		Duration of treatment		Quality of control		Insu- lin shock	Throm- bocytes per cu. mm. of blood	Remarks
						Fast- ing	Max.†	Chlor- prop- amide gm./ day	Insulin U./day	Chlor- prop- amide (weeks)	Insulin (months)	Chlor- prop- amide	Insu- lin			
53.	N702	Ind.	M	43	42	160	362	1.5	SI 40 PZI 48	5	11	poor	good	nil	—	chlorpropamide discontinued
54.	N1034	Ch.	M	64	60	—	—	0.75	SI 40 PZI 24	20	5½	good	good	coma once	110,000	retinopathy and cataract
55.	N2123	Ind.	M	39	33	280	362	0.25	IZS(L)28	18	2	good	poor	nil	165,000	random blood sugar: 93 mg. %
56.	N2386	Ind.	M	51	50½	206	388	1.0	IZS(L)80	9	5	good	poor	coma once	140,000	insulin gradu- ally withdrawn
57.	N668	Ind.	M	45	44	174	312	1.0	IZS(L)136	20	9	good	fair	nil	160,000	random blood sugar: 111 mg. %
58.	N1873	Ch.	M	39	39	146	—	1.0	IZS(L)24 IZS(SL)24	13	2	good	fair	nil	150,000	random blood sugar: 111 mg. %
59.	2134/57	Malay	M	48	45½	166	332	0.75	IZS(L)44	9	2½	good	fair	nil	150,000	random blood sugar: 86 mg. %
60.	N1724	Malay	M	33	30	241	326	1.0	PZI 28	9	—	good	fair	nil	200,000	random blood sugar: 156 mg. %
61.	935/56	Ind.	M	61	55	—	—	1.0	SI 52 PZI 28	9	5 yrs.	good	fair	nil	—	random blood sugar: 139 mg. %
62.	N989	Ind.	M	45	42½	184	302	0.5	IZS(SL)40 IZS(L)44	11	18	good	good	nil	140,000	random blood sugar: 77 mg. %
63.	1013/57	Malay	M	34	33	180	280	0.5	IZS(L)56	11	11½	good	fair	nil	195,000	random blood sugar: 77 mg. %
64.	N497	Malay	F	51	48	303	454	0.25	IZS(L)32	9	36	good	good	nil	165,000	—
65.	1907/56	Ind.	M	67	60	120	255	0.75	PZI 52	8	18	good	good	nil	150,000	—
66.	939/56	Ind.	F	42	33	147	326	1.0	IZS(L)72	5	39	fair	poor	nil	—	—
67.	1819/56	Ind.	F	59	57	200	332	1.0	PZI 24	5	5	fair	good	nil	140,000	—

* Key: Ind., Indian; Ch., Chinese.

† In glucose tolerance test.

patients thrice daily—before breakfast and after lunch and dinner. Patients were given full clinical examination whenever they complained of any symptoms that might be construed as probable side effects of the drug, and they were questioned directly for any symptoms referable to the cardiovascular, digestive, urinary, and neurological systems and about any skin reaction.

Patients who were on insulin or tolbutamide, regardless of the quality of control achieved, had their insulin or tolbutamide discontinued for a variable

TABLE 6
CORRELATION BETWEEN AGE AT ONSET OF DIABETES AND CONTROL ACHIEVED

Quality of control	No. of patients per age group*									Total
	26-30	31-35	36-40	41-45	46-50	51-55	56-60	61-65	66-70	
Good...	1 (50)	6 (85.7)	6 (60.0)	9 (75)	13 (85.6)	9 (69.2)	3 (75)	1 (50)	1 (50)	49 (73.1)
Fair...	1 (50)	1 (14.3)	3 (30)	2 (16.6)	1 (7.2)	2 (15.4)	1 (25)	nil	nil	11 (16.4)
Poor...	nil	nil	1 (10)	1 (8.4)	1 (7.2)	2 (15.4)	nil	1 (50)	1 (50)	7 (10.5)
Total..	2 (100)	7 (100)	10 (100)	12 (100)	15 (100)	13 (100)	4 (100)	2 (100)	2 (100)	67

* Figures in parentheses are percentages of total.

period until glycosuria indicated a relapse of the diabetic state. Chlorpropamide was then given in place of insulin or tolbutamide.

Dosage of chlorpropamide. As the full toxicology of chlorpropamide in man is not known, it was decided to start treatment with the drug in a relatively small dosage given once or twice daily for the first week. Initially, 0.5 gm. of the drug was given, but this was reduced to 0.25 gm., the smaller dose being less likely to produce side effects. The dosage of 0.25 gm. was increased at weekly intervals to 2, 3, or even 4 times daily until control was achieved or until side effects necessitated the discontinuance of treatment. Once control was established, the dosage was reduced to the maintenance level, as adjudged by the relative absence of glycosuria.

Classification of clinical data. Taking into consideration the renal threshold for glucose in the individual patient, the quality of control of the diabetes was assessed on the basis of the degree of glycosuria present. A condition with no sugar in the urine, or only infrequent traces of sugar was designated good. Where traces of sugar in the urine occurred frequently, after dietary deviations had been excluded, the control of the diabetes was classified as fair. Greater glycosuria constituted poor control. The clinical data are presented in TABLES 3, 4, and 5.

TABLE 7
CORRELATION BETWEEN SEX AND RACE AND CONTROL ACHIEVED

Quality of control	Sex		Race				Total
	Male	Female	Chinese	Indian	Malay	Others	
Good.....	34 (80.9)	15 (60)	16 (64)	23 (74.2)	7 (87.5)	3 (100)	49 (73.1)
Fair.....	4 (9.5)	7 (28)	4 (16)	6 (19.4)	1 (12.5)	nil	11 (16.4)
Poor.....	4 (9.6)	3 (12)	5 (20)	2 (6.4)	nil	nil	7 (10.5)
Total.....	42 (100)	25 (100)	25 (100)	31 (100)	8 (100)	3 (100)	

* Figures in parentheses are percentages of total.

TABLE 8
CORRELATION BETWEEN DOSAGE OF CHLORPROPAMIDE AND CONTROL ACHIEVED

Quality of control	No. of patients per chlorpropamide dosage, gm.*					Total
	0.25	0.5	0.75	1.0	1.5	
Good.....	7 (10.4)	17 (25.4)	14 (20.9)	11 (16.4)	nil	49 (73.1)
Fair.....	nil	3 (4.4)	4 (6)	4 (6)	nil	11 (16.4)
Poor.....	nil	1 (1.5)	2 (3.0)	3 (4.5)	1 (1.5)	7 (10.5)
Total.....	7 (10.4)	21 (31.3)	20 (29.9)	18 (26.9)	1 (1.5)	67 (100)

* Figures in parentheses are percentages of total.

Analysis of clinical data. Analysis of the clinical data reveals that, of the 67 patients treated with chlorpropamide, 49 (73.1 per cent) showed the diabetes (glycosuria) under good control. In 11 patients (16.4 per cent) the control was fair, and in 7 (10.5 per cent) the control was poor.

Correlation between the quality of control achieved under treatment and any one of the following factors—age at onset of diabetes, race, sex, and dosage of chlorpropamide used—is given in TABLES 6, 7, and 8.

Discussion

Hypoglycemic activity of chlorpropamide. There is no doubt that chlorpropamide is an effective hypoglycemic agent, as it was able to reduce glycosuria to a minimum in 73.1 per cent of the cases studied. This segment is made up of the 49 good-control patients, whose ages at the onset of diabetic symptoms ranged from 30 to 66 years. The degree of control of the diabetes, when related to the ages of the patients at the onset of diabetic symptoms (TABLE 6), does not reveal with any certainty that the drug is more effective in the older than the younger age group; 85.7 per cent of patients between the ages of 31 and 35 achieved good control of glycosuria, while 75 per cent between the ages of 56 and 60 also had good control.

TABLE 9
CORRELATION BETWEEN FASTING BLOOD SUGAR AND CONTROL ACHIEVED

Quality of control	No. of patients per fasting blood sugar levels, mg. per cent*		
	50-150	151-250	251-350
Good.....	5 (55.6)	29 (76.3)	7 (77.7)
Fair.....	2 (22.2)	5 (13.1)	2 (22.3)
Poor.....	2 (22.2)	4 (10.6)	nil
Total.....	9 (100)	38 (100)	9 (100)

* Figures in parentheses are percentages of total.

The data of TABLE 7, where the sex of the patients is related to the degree of control of the diabetes, shows that 80.9 per cent of male and 60 per cent of female patients achieved good control. It would seem that chlorpropamide is somewhat more effective in male than in female diabetics, but a definite pronouncement on this problem cannot be made without further investigation in a larger series of cases.

In relating the ethnic groups of patients to the number of cases with good control of glycosuria, it is obvious that chlorpropamide is equally effective in Chinese, Indians, and Malays.

Chlorpropamide dosage. The dosage of chlorpropamide required to maintain good control ranged from 0.25 gm. to 1.0 gm. daily, with an average total daily requirement of 0.647 gm.

The majority (31 patients) of good-control cases required a maintenance dose of 0.5 to 0.75 gm. daily, these cases constituting 46 per cent of the entire series and 63 per cent of all good-control cases. With the exception

of 1 patient in the poor-control group, to whom a daily total dose of 1.5 gm. of chlorpropamide was given, it is noteworthy that the total daily drug dosage in the fair-control and poor-control groups ranged from 0.5 to 1.0 gm.

It may be that further increase in the drug dosage for these two groups will reduce further the glycosuria of a few patients.

Correlation studies between the fasting blood sugar level and the quality of control achieved under treatment do not indicate that patients with a high

TABLE 10
CORRELATION OF FASTING BLOOD SUGAR TO CHLORPROPAMIDE
DOSAGE IN GOOD-CONTROL CASES

Chlorpropamide dosage, gm.	No. of patients per fasting blood sugar levels, mg. per cent*			Total
	50-150	151-250	251-350	
0.25.....	1 (14.2)	3 (11.1)	2 (28.4)	6
0.5.....	4 (57.4)	9 (33.3)	3 (43.2)	16
0.75.....	1 (14.2)	9 (33.3)	1 (14.2)	11
1.0.....	1 (14.2)	6 (22.3)	1 (14.2)	8
Total.....	7 (100)	7 (100)	7 (100)	41

* Figures in parentheses are percentages of total.

fasting blood sugar level necessarily required higher dosage to attain good control of their glycosuria. Neither does a low fasting blood sugar level indicate a low-dosage requirement to obtain good control. Thus, the fasting blood sugar range of good-control cases is 86 to 350 mg. per cent, and 27 of these 41 patients (65.8 per cent) required a maintenance dosage of 0.5 to 0.75 gm. daily. Correlation between the fasting blood sugar level and the quality of control achieved under treatment is shown in TABLE 9; the relationship between the fasting blood sugar level and the dosage of chlorpropamide required to achieve good control is shown in TABLE 10.

Patients previously on tolbutamide therapy. In Group B (TABLE 4) of this trial series, 25 patients were treated with chlorpropamide after glycosuria returned or became more severe when tolbutamide therapy was discontinued. In this group, 9 patients (36 per cent of the group) were subsequently better controlled on chlorpropamide than on tolbutamide, and 4 (16 per cent) were not as well controlled as with tolbutamide. The remaining 12 patients (48 per cent) had as good control with chlorpropamide as with tolbutamide.

Included in the 4 cases (Nos. 28, 32, 41, and 44) in which chlorpropamide was less effective than tolbutamide was 1 patient (No. 28) who was given chlorpropamide for 2 weeks, at which time he refused to continue treatment with the drug because of side effects. Control was subsequently re-established with tolbutamide.

A comparison of the dosage of chlorpropamide with that of tolbutamide in patients whose glycosuria was controlled to a similar degree shows the average dose to be 0.66 gm. for chlorpropamide and 2.0 gm. for tolbutamide. Thus, weight for weight, chlorpropamide is a more effective hypoglycemic agent than tolbutamide.

Patients previously on insulin therapy. Patients on insulin therapy before chlorpropamide treatment constitute Group C (TABLE 5) of this series. Of the 15 patients of this group, 9 (60 per cent) achieved substantially better control of their diabetes than on insulin treatment. The insulin dosage ranged from 28 to 136 U. daily, with an average of 63.5 U.; the average dose of chlorpropamide was 0.83 gm. In 4 patients (26.6 per cent) chlorpropamide did not reduce the glycosuria any more than did the insulin given previously. In the remaining 2 patients (Nos. 53 and 67) treatment with chlorpropamide resulted in poorer control than with insulin. In 1, the chlorpropamide dosage was increased to 1.5 gm. daily while, in the other, the dose was increased to 1.0 gm. daily. In both cases, the chlorpropamide was subsequently withdrawn and insulin resumed; good control was re-established.

Side effects of chlorpropamide. Throughout the study with chlorpropamide, a close watch was kept for any side effects that might be considered as due to the drug action. Of the 67 trial cases, 39 (58.2 per cent) had no side effects of any description, even on direct inquiry. Five patients (7.5 per cent) reacted strongly to the drug (Nos. 17, 28, 41, 44, and 53), and severe giddiness, fatigue, anorexia, constipation, nausea, and vomiting necessitated discontinuance of chlorpropamide. The remaining 23 patients (34.3 per cent) had mild side effects, most of which disappeared spontaneously even when the drug was continued, frequently with increased total daily dosage. The distribution of the mild symptoms complained of by the 23 patients (some of whom complained of more than one symptom) follows:

Symptoms	Number affected
Constipation:	10
Giddiness:	7
Skin rash:	4
Anorexia:	4
Fatigue:	2
Epigastric pain:	1
Profuse sweating:	1

Constipation was the most frequent complaint and, apart from a decrease in the frequency of bowel action, patients stated that evacuation seemed incomplete and unsatisfying. A few resorted to laxatives, but in the majority the constipation declined gradually.

Giddiness or dizziness was a not infrequent complaint soon after therapy was started. The symptom was not accompanied by others referable to

hypoglycemia, it was not dispelled by food, and there was no hypoglycemia found upon blood sugar analysis. There was some suggestion that the size of the initial drug dosage contributed to the occurrence of the dizziness.

Of the 4 patients in whom skin rashes developed (Nos. 32, 34, 35, and 46), 3 had scattered, symmetrical, mildly itching erythematous eruption, which took about 5 to 7 days to subside. This erythematous rash did not reappear in the same patient even when the drug dosage was increased subsequently. In the fourth patient the rash was purpuric and fairly profuse, and a thrombocyte count, done 3 to 4 days after the purpuric spots and patches appeared, registered 180,000 per cubic millimeter of blood. This rash also subsided gradually without reappearance.

Hematology and urinalysis. There was no appreciable change in the RBC and WBC counts in the patients during treatment with chlorpropamide. Thrombocyte counts were done on 45 patients at intervals; in 8 patients (22.2 per cent) the counts registered values at and below 100,000/cu. mm. of blood, but none suffered from bleeding. The majority, comprising 31 cases (68.9 per cent), had counts ranging from 100,000 to 200,000/cu. mm. of blood. The remaining 6 cases (17.8 per cent) had counts higher than 200,000/cu. mm. It seems that chlorpropamide can produce a fairly persistent reduction in the thrombocyte count although, in the single patient who had a purpuric rash (No. 34), the count was 180,000/cu. mm. of blood.

No abnormality was found when the urine of patients on chlorpropamide therapy was repeatedly examined at intervals for albumin, RBC, WBC, casts, and crystals.

Conclusions

Chlorpropamide is an effective hypoglycemic agent and, in this respect, it produced good control of the diabetes in 73.1 per cent of the cases studied.

Chlorpropamide is as active in the younger patients (31 to 35 years) as in the older (56 to 60 years).

There is some evidence that the drug is more effective in male diabetics than in female diabetics.

The hypoglycemic action of chlorpropamide is the same regardless of the racial characteristics of the patients studied.

Weight for weight, chlorpropamide is a more efficient hypoglycemic drug than tolbutamide, 0.66 gm. of chlorpropamide being required for maintenance of good control as against 2 gm. of tolbutamide.

There is no indication that, to effect good control of the diabetes, chlorpropamide dosage need be proportional to the height of the fasting blood sugar level.

The maintenance dosage of chlorpropamide is between 0.5 and 0.75 gm. per day.

In this study of patients with sthenic-plethoric bodily configuration, chlorpropamide treatment yielded a higher percentage and better quality of control of the diabetes than did insulin therapy.

Chlorpropamide may produce undesirable side effects, which are frequently mild and subside spontaneously.

There is evidence that chlorpropamide has a mild to moderate thrombocytopenic action.

Acknowledgment

I thank Government Office of Medical Services, Singapore, for permission to publish this paper, and Chas. Pfizer & Co., Brooklyn, N. Y. for supplying the chlorpropamide used in this trial.

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CLINICOTHERAPEUTIC EVALUATION OF CHLORPROPAMIDE, A NEW SULFONYLUREA DERIVATIVE FOR THE TREATMENT OF DIABETES MELLITUS

Pedro Serrano and Rafael Chavez

National Institute of Cardiology, and Hospital Colonia, Mexico, D. F., Mexico

Early pharmacological work indicated that chlorpropamide had a higher potency than other sulfonylurea derivatives, namely carbutamide and tolbutamide. The objective of the study reported here was to confirm such findings from a clinical point of view in human patients with diabetes mellitus.

The diabetic patients in whom chlorpropamide was assayed, were laborers of the National Railways of Mexico; almost all were traveling workers and, for that reason, required ambulatory treatment. Only patients with uncomplicated diabetes were chosen and none was alcoholic. Selection was made regardless of the activity of the diabetic process, onset of the disease, age, sex, or constitution of the patient. This was done in order to compare the results with those reported in using the earlier antidiabetic drugs, which had proved suitable for treating overweight, middle-aged patients who had mild or moderately active diabetes and were free to develop acidosis.^{1-5, 10, 14}

Periodic control during the treatment was performed in 33 subjects for 2 to 6 months; control was observed by daily glycosuria tests (3 times a day) and routine clinical and laboratory examinations. Of the 33 cases studied, 19 were submitted to special weekly laboratory tests including urine analysis, blood chemistry, RBC, WBC, and liver function tests. These tests were chosen in order to detect changes reported as occurring during treatment with carbutamide or tolbutamide (such changes as a decrease in hemoglobin concentration, agranulocytosis, and alterations of the RBC or WBC as described in experimental and clinical works).^{6-9, 12} Liver damage is probably the most important complication of the treatment with oral antidiabetic drugs;⁷⁻¹³ for this reason, in several patients, a battery of liver function tests was done, including thymol turbidity, thymol flocculation, cephalin flocculation, and serum bilirubin. The routine blood chemistry tests included those for glucose, urea, and cholesterol, as well as a glucose tolerance test, which was done in some patients.

The amount of chlorpropamide to be given to each patient was calculated as follows. When no previous treatment had been given, 1 gm. of the drug was allowed in patients with glycemias from 250 to 350 mg. per cent; when insulin had been administered, 40 to 60 U. were considered equivalent to 1 gm. of chlorpropamide; when oral sulfonylureas constituted the previous treatment, 1 gm. of chlorpropamide was administered instead of 1.5 gm. of the other drug. Chlorpropamide was used at a dosage of 1.5 gm. only in patients who had been treated with more than 60 U. of insulin daily, or when a combination of insulin plus oral hypoglycemic drugs was necessary.

The evaluation of the results was made according to the grade of the diabetic process, as well as by comparison of the laboratory analyses performed before and after treatment with chlorpropamide.

Clinical Results

The clinical results were classified excellent when complete insulin substitution was achieved with satisfactory control and no observed complications; good when satisfactory control was achieved without complete insulin substitution or when temporary intolerance was observed; and unsatisfactory when a persistent intolerance was present or when insulin requirements could not be decreased.

Of the 33 patients (2 females and 31 males, 33 to 69 years old), only 5 had severe diabetes and 3 were underweight (TABLE 1). Twenty were designated as giving excellent results; 10, good results; and only 3, unsatisfactory results.

The hypoglycemic effect of the drugs was observed in every patient; 2 had hypoglycemia (demonstrated by blood glucose determination), and many showed temporary intolerance, especially at the beginning of the treatment. The latter symptoms, which disappeared with decrease of the drug dosage, were probably due to an early and strong hypoglycemic effect. In the former 2 cases of hypoglycemic precoma, glucose was administered intravenously, but the release of the symptoms was observed only after the drug had been discontinued several hours.

The final maintaining doses were: 125 mg. in 4 patients; 250 mg. in 6 patients; 500 mg. in 9 patients; 750 mg. in 1 patient; and 1 gm. in 10 patients. Only in 3 unsatisfactory cases were the doses increased to 2.5 gm. a day before discontinuation. In 5 patients, the drug was discontinued and neither glycosuria nor hyperglycemia has been observed during 1 to 2 months.

Insulin was substituted completely in 3 patients in whom it had been the previous treatment; in 1 of these patients 250 mg. was sufficient to fill the former requirement of 80 U. of insulin daily. In 9 cases of combined treatment (insulin plus tolbutamide) the complete replacement of insulin was achieved in 7 patients (superseding former insulin requirements of 15 to 40 U. daily) with chlorpropamide in the same amount as the former anti-diabetic drugs. There were exceptions in 2 cases, in which this was reduced still further, from 1 gm. of tolbutamide to 0.5 gm. of chlorpropamide. In the other cases of combined treatment, the insulin requirement was reduced to 20 U. daily maintaining, gram for gram, the administration of chlorpropamide instead of the previous sulfonylurea.

In 16 cases in which oral hypoglycemic drugs were the previous medication, only 1 patient required the same amount of chlorpropamide as tolbutamide; in the other 15 cases, the average reduction was from 1.11 gm. of other sulfonylureas to 0.445 gm. of chlorpropamide, which represents an approximate 60 per cent reduction. In every case, the change from tolbutamide to chlorpropamide was made easily.

Of the 20 cases of excellent results, almost all were overweight patients and none had severe diabetes. Only 2 of the 10 cases with good results had

TABLE 1
RESULTS OF TREATMENT WITH CHLORPROPAMIDE

Excellent										
General data						Previous treatment		Treatment with chlorpropamide		
No.	Age	Sex	Evolution time (years)	Type of diabetes	Constitution %*	Insulin	Oral anti-diabetic drugs	Insulin (U./day)	Chlorpropamide (gm./day)	Duration of treatment (months)
1	44	M	2	Slight	+10		†1.00		0.500	1
2	49	M	17	Moderate	Normal		†1.25		0.250	2
3	50	M	11	Moderate	+10	‡20	1.00		1.000	2
4	54	M	4	Moderate	-20		1.00		0.125	3†
5	54	F	4	Moderate	+20	30	1.00		1.000	2
6	55	M	1	Slight	+20				0.125	1†
7	55	M	5	Slight	+20		1.00		0.500	3
8	56	M	22	Moderate	+20	30			0.500	1
9	57	M	1	Moderate	+10		†1.00		0.125	6†
10	57	M	4	Moderate	+10		†1.50		1.000	1
11	60	M	1	Moderate	+10		0.50		0.250	2
12	61	M	6	Moderate	+20	‡30	1.00		1.000	1
13	62	M	5	Moderate	Normal	20	1.00		0.500	1
14	62	M	6	Moderate	Normal		†1.00		0.125	3†
15	63	M	4	Moderate	+10	40	1.00		1.000	2
16	63	M	5	Slight	+30		1.50		1.000	1
17	63	M	6	Moderate	+20	‡30	1.00		1.000	1
18	63	M	6	Moderate	+20	30			0.500	2
19	66	F	10	Slight	+20		†1.00		0.250	1
20	69	M	19	Moderate	Normal		1.00		0.500	2
Satisfactory										
21	38	M	3	Moderate	Normal		†1.50		0.250	2
22	52	M	4	Moderate	+20	‡80			0.250	3
23	54	M	4	Moderate	+20		‡2.00		0.500	1
24	58	M	18	Slight	Normal				0.750	1
25	59	M	14	Severe	Normal	40	1.00	20	1.000	6
26	60	M	4	Moderate	Normal		†1.00		1.000	1
27	62	M	5	Severe	+20	‡60	1.00	40	1.000	2
28	62	M	20	Moderate	+10		†1.00		0.500	1
29	68	M	13	Moderate	+20	‡15	1.00		0.500	6†
30	68	M	22	Moderate	Normal		‡0.50		0.250	2
Unsatisfactory										
31	33	M	8	Severe	-20	‡60		30	2.500	1
32	52	M	12	Severe	-20	40		40	1.500	1
33	63	M	2	Severe	+10	60		60	1.500	1

* Constitution given in increase (+) or decrease (-) according to patient's ideal weight.

† Cases in which the daily requirements of chlorpropamide decreased to zero, being controlled in one to two months.

‡ Requirements are more than stated because of incomplete control of the case.

severe diabetes and all had a normal weight or were overweight. The 3 cases of unsatisfactory results had severe diabetes; 1 was underweight, well controlled previously with 80 U. of insulin daily, and could not reduce his insulin requirements with as much as 1.5 gm. of chlorpropamide. Of the remaining 2, one was underweight, being the youngest subject of the study (33 years old), and both were psychopathic. These last 2 cases showed persistent

gastrointestinal intolerance to the drug, and no reduction of the insulin requirements could be achieved.

The side effects observed during the treatment with chlorpropamide were almost always moderate with only transitory intolerances, probably due to overdoses of the drug. Gastrointestinal disturbances such as hiccough, gastric pain, or diarrhea were present in six cases and were of important intensity in only two. In every patient the symptoms disappeared with the reduction or withdrawal of the drug. Nervous-system disturbances such as nausea or vertigo, appeared in two cases, disappearing with the temporary discontinuation of the drug. There was only one case of dermatological reaction; this consisted of papula on the head of the patient and was a moderate reaction, lasting a short time and disappearing with the reduction of the drug dosage. Three other patients (one not included in these thirty-three cases), observed a generalized peripheral vasodilatation, especially over the face and neck. This reaction, of variable intensity, was originated by the ingestion of a cup of wine or glass of beer; the test was made with other patients, but the effect could not be reproduced.

Laboratory Data

Unimportant changes were detected by routine urine analysis; no hematuria, cells or casts appeared during administration of the drug. A slight albuminuria was present in some of the patients; this did not increase significantly in any case and, in some, was even lower than in the initial control period of the study. This effect was probably due to a better control of the altered metabolic situation.

A decrease in red blood cells observed in 7 patients was less than 1 million in each of 6 and, in one case, the decrease was from 5,100,000 to 4,100,000. Microcytic hypochromic anemia was present in 2 patients before treatment and did not show any modification during the time the drug was given; 5 of the 7 patients mentioned showed a tendency to hypochromic anemia. In the rest of the cases, the RBC was practically unchanged. No important change was found in the WBC or in the total leukocyte or differential counts.

Total cholesterol was normal or slightly increased before treatment. No important modification could be found in this determination. Blood urea, slightly increased (37 to 44 mg. per cent) in 6 cases, was normal, after treatment in every case except 3 of the previous 6 mentioned; in these a slight further increase was found (44 to 55 mg. per cent).

One to 3 liver function tests were performed before and after treatment on each of the 19 subjects; thymol turbidity was done in 16 cases; cephalin flocculation in 15; serum bilirubin in 11; and a Bromsulphalein test in 3. No damage was demonstrated by these liver function tests. In 7 cases in which thymol turbidity was slightly increased before the treatment with chlorpropamide (from 6 to 14 U.), it was found to be within the normal values after treatment.

In the two cases in which glucose tolerance tests were done, the general morphology of the test was practically unchanged before and after treatment.

Conclusions

The new compound chlorpropamide showed its hypoglycemic effect in every case. Excellent or satisfactory results were observed in 28 cases of moderate or slight diabetes; unsatisfactory results were reported for 3 patients with severe diabetes; and 2 cases of severe diabetes gave satisfactory results. The indications and contraindications of this drug seem to be the same that have been reported for carbutamide and tolbutamide.¹

The toxic effects seem to be similar to those reported for the other anti-diabetic drugs; the observed lack of response to glucose administration was similar to that reported in animals by Houssay.¹⁵

Although these drugs seem to have no effect on the metabolism of alcohol,¹⁶ the ingestion of it produced important peripheral vasodilatation in three cases; this effect was not accompanied by hypertension or headache and lasted for two to four hours.

No toxic effect could be shown by the liver function tests; such findings are difficult to evaluate because of the liver dysfunction that is frequently present in treated or untreated cases of diabetes mellitus.¹⁷⁻¹⁹

The potency of the drug seems to be 2 to 3 times that of carbutamide or tolbutamide but has individual variation from case to case; some patients are well controlled with 0.125 gm. of chlorpropamide while others require as much as 1 or 1.5 gm. in order to achieve correct control.

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CLINICAL STUDIES WITH CHLORPROPAMIDE

Edward Tolstoi

New York Hospital-Cornell University Medical College, New York, N. Y.

Fifteen office patients with diabetes were treated with chlorpropamide. In 6 it was necessary to discontinue the drug: 4 developed ketosis; 2 lost considerable weight and had poor appetites, although their blood sugars approached normal values and there was no glycosuria; 9 are still under observation. The shortest period of follow-up was 2 months; the longest, 6. The ages of the 4 men and 5 women treated ranged from 35 to 70. All but 1 are diabetics who had been on insulin and who, at other times, managed their diabetes by dietary means, thus providing the factors necessary for a comparison.

All of these patients were permitted a normal or usual diet. They neither weighed nor measured their food, and they had sweets on occasion. At first they were seen every other day, then at weekly intervals, and now at monthly visits. The dosage of chlorpropamide varied from 500 to 1000 mg. At each visit the patients were questioned as to symptoms of diabetes or hypoglycemia (or both), pruritus, skin eruptions, or weakness; later they were also questioned as to loss of appetite. Body weights were recorded, blood pressures taken, and eye grounds studied. The urines were examined for albumin, sugar, and acetone when indicated. Tests were made for blood levels of sugar and chlorpropamide* and, on occasion, blood counts and some constituents reflecting liver function were also determined.

At first the immediate response to the drug was tested. The 5 patients used were 30 to 60 years old, and had had diabetes from 1 month to 14 years. All came to the office without breakfast and, after blood was drawn and a urine specimen obtained, 1 gm. of chlorpropamide was administered to each. One hour later, blood and urine samples were obtained, and the patients were then sent out for a breakfast consisting of fruit juice, 2 eggs, 1 slice of toast, and a beverage; some had cereal instead of the eggs. The blood and urines were examined two hours after this breakfast, and a fasting specimen was taken 24 hours after the initial administration of the drug.

From TABLE 1 it is clear that only 2 of the 5 unselected patients showed a hypoglycemic reaction to the test dose. Patient W. Mc. showed a consistent fall in the blood glucose, the effect persisting overnight. The chlorpropamide blood levels were constant, and only a slight drop was seen after 24 hours. With so definite a response, this patient appeared suitable for therapy with chlorpropamide. However, since he was obese and his diabetes was of only 1 month's duration, he was treated by subcaloric diet only, no drug being given. He lost weight slowly and, with this weight reduction, his glycosuria cleared. He has no symptoms and his last fasting blood sugar was 130 mg. per cent. Had we given him chlorpropamide with the prescribed diet, we certainly would have considered the result a successful

* Chlorpropamide levels were determined by Chas. Pfizer & Co., Inc., Brooklyn, N. Y.

response to the drug. Patient R. B. has had diabetes for 4 years. His symptoms were inconstant and his glycosuria, although occasional, was most apparent postprandially. He weighed 215 lb. and, 4 years ago, dietary therapy alone was advised. On a subcaloric diet he lost weight; with the loss of 30 lb., however, he also had symptoms of diabetes, with intense fatigue as the most troublesome feature. He refused insulin, and chlorpropamide seemed ideal for him. His hypoglycemic response showed the test dose to be a failure, even though the drug's blood level was sustained. His firm refusal to use insulin, however, prompted us to try chlorpropamide. He

TABLE 1
DATA ON SIX PATIENTS TREATED WITH CHLORPROPAMIDE

Patient	Sex	Age	Duration of diabetes	NPH Insulin, U.	Blood sugar, mg. per cent*				Chlorpropamide blood levels, $\mu\text{g.}/\text{ml.}$			
					Fasting	1 hour after 1.0-gm. dose	2 hours after break-fast	After 24 hours	Fasting	After 1 hour	After 3 hours	After 24 hours
W. Mc.	M	50+	1 month	0	215 S ++	192 S +	158 S +	134 S 0	0	94	97	87
R. B.	M	50+	4 years	0	308 S 4+	294 S 3+	286 S 4+	265 S 4+	0	8?	93	81
H. J.	M	40+	3 years	10	210 S +	180 S \pm	104 S 0	153 S +				
J. D.	F	30+	10 years	50	330 S 4+ AC +	330 S 4+ AC 3+	440 S 4+ AC 4+	Terminated R25	0	46	64	—
S. R.	M	60	14 years	60	357 S 4+ AC 0	344 S 4+ AC 4+	395 S 4+ AC 4+		0	33	24	59† 50†
E. C.	F	60	2 years	0	360 S 4+		360 S 4+					

* Lower figure or figures: S represents urine sugar; AC, acetone.

† Results are for same sample, split.

was given 1.0 gm. daily; surprisingly, the glycosuria cleared, most of his symptoms of diabetes disappeared and, for 2 months, his fasting blood sugars ranged from 170 to 200 mg. per cent. The liver function tests were normal, as was the hemogram. The chlorpropamide level was 318 $\mu\text{g.}/\text{ml.}$ All of this was spectacular but, although he ate well, he did not enjoy his food and was losing weight at the rate of 5 lb. per month. Because we suspected some unpleasant complication he was hospitalized, but an extensive study failed to reveal any abnormalities. While he was in the hospital the chlorpropamide was continued and he chose his own foods. Although all fractional urine specimens were sugar-free, and the blood sugars were 125 and 132 mg. per cent, he felt tired, did not enjoy his food, and failed to gain weight even though he forced himself to eat his meals. Failure to improve

while in the hospital convinced this patient that the therapy was inadequate, and he accepted the idea of insulin therapy. The change produced by 20 U. of NPH insulin was dramatic; he feels well, enjoys life, and has no complaints. He has gained weight and is maintaining it.

The third patient, H. J., is an archaeologist whose diabetes required only 10 U. of NPH insulin to keep him free of symptoms and at his optimum weight; his diet was normal. Since he was going on an expedition, and refrigerating the insulin might be a problem, I thought that chlorpropamide would be the ideal solution, particularly since his response to the test dose was so definite. He took along a supply of the medication, but, on return, he told me that he had been compelled to discontinue it, because after 2 weeks his appetite failed, he lost weight, and felt weak. His urine specimens were sugar-free. As he did not do well clinically he resumed taking insulin and showed a prompt and definite improvement.

In patients J. D. and S. R., the experiments were terminated because of ensuing ketosis. Two other patients developed ketosis when the insulin was reduced; chlorpropamide was then discontinued.

Patient E. C. also showed no response to a test dose, but with a 1.0-gm. dose of chlorpropamide the blood sugar fell to 138 mg. per cent, and the urine remained sugar-free, even though she was on a self-selected, nonweighed, and unmeasured diet, including occasional sweets. This patient at one period was treated by diet only, and she did not do as well with respect to her glycosuria as she did on chlorpropamide. Unfortunately, the experiment could not be continued because she suffered a nonrelated cerebrovascular accident and died.

The 9 patients treated with chlorpropamide experienced no untoward effects. Some of these patients have taken 10 to 20 U. of insulin for considerable periods; at times they had been treated by diet only. Insulin had been used to maintain or increase the patient's weight when indicated. When chlorpropamide therapy was instituted, no change was made in the diets and, although these were supplemented with occasional sweets, the blood sugar continued to fall. Although the blood sugars did not usually descend to normal values, the patients showed no symptoms, the glycosuria decreased or cleared, and weight was not only maintained but increased. Such liver function tests as total protein, serum albumin, and alkaline phosphatase were within normal limits, as was the hemogram. The urines showed no abnormalities of renal function.

Rather than burden this short paper with case histories of these patients I shall include only limited data (TABLE 2) that are representative of the small group examined. The table shows that the patient was treated with chlorpropamide for 6 months. I began with 1.0-gm. dose and, later, when he complained of a "metallic" taste, lowered the dosage to 0.7 gm. It is clear that his fasting blood sugar values, although not normal, are lower, and neither his weight nor the chlorpropamide blood levels have varied significantly. There are no chemical evidences of liver function impairment. The patient is living normally, is in good condition clinically, and has no complaints.

From my data of this small, but closely observed group of patients, certain

conclusions are valid. It is clear that chlorpropamide lowers the blood sugar in selected patients with diabetes. As a consequence, the glycosuria also decreases or clears. These phenomena occur when the patients are on a normal diet, exclusive of concentrated sweets, or have sweets on occasion only. The same patients on similar diets, without chlorpropamide, revealed both a hyperglycemia and a 1- to 2-plus glycosuria. To correct this, it was necessary to restrict the diet. Thus, a 500- to 1000-mg. dose of chlorpropamide enabled a certain group of patients to eat more liberally, keep the urine sugar-free, and maintain (or gain) weight.

It must be pointed out, however, that although a fall in the blood glucose follows the administration of chlorpropamide, some patients not only fail to

TABLE 2
DATA ON PATIENT L. C., 49 YEARS OLD, DIABETIC 8 YEARS

1958	Weight	Blood sugar mg. %	Urine sugar	Chlorpropamide		Total protein	Albumin	Alkaline phosphatase
				Dosage mg.	Level μg./ml.			
4/16	178	250	2+	1000		7.0	4.8	2.8
4/23	180	200	1+	1000	264			
5/8	177	176	0	1000	273			
5/22	176	176	0	1000	326			
6/5	174	170	0	700	257	7.0	4.4	2.7
6/18	172	148	0	700	269			
7/3	173	150	0	700	262			
8/13	174	180	0	700	256			
9/17	174	150	0	700	207			
10/23	175	160	0	700				

gain, but continue to lose weight. This circumstance was observed in two of our patients, and should focus attention on the fact that lowering of the blood sugar is not synonymous with the satisfactory treatment of diabetes and that all one is justified in claiming for chlorpropamide thus far is that it lowers the blood sugar in selected patients who, if they are not obese, may liberalize their diets.

The following conclusions may be drawn from this study.

Chlorpropamide lowers the blood sugar in selected patients.

A test dose is not helpful in determining the patient's response to continued therapy.

There is no relationship between the chlorpropamide blood level and the hypoglycemic effect of the dosage used.

A poor appetite is a bad prognostic sign, regardless of the blood sugar level.

The drug maintained the weight and kept certain patients aglycosuric on normal diets; this had not been accomplished without chlorpropamide in the same patient.

Treatment of the blood sugar only does not constitute treatment of the totality of the underlying defects in the metabolism of diabetes mellitus.

THE USE OF CHLORPROPAMIDE IN BRITTLE AND POORLY CONTROLLED DIABETES MELLITUS*

Harold L. Dobson, Hooshang Guilak,† R. E. Carter,†
Hunter Montgomery,† James A. Greene

Baylor University College of Medicine and Jefferson Davis Hospital, Houston, Tex.

The development of the oral hypoglycemic agents has led to some sweeping revisions in concepts about the control of the abnormal carbohydrate metabolism in diabetes mellitus. With the compounds presently available (tolbutamide in the United States and tolbutamide and carbutamide in Europe), oral therapy is available only to patients with mild, stable, adult-type diabetes.¹⁻³ It is well known that these patients are controlled by proper diet alone or by diet with small doses of insulin. Consequently use of the oral agents for these patients has been more a convenience than a necessity. Patients in urgent need of some agent that will provide more stable control are the juvenile diabetics and the adult diabetic patients who are poorly controlled on any dose of insulin. Included in the latter group are those patients who are brittle in the sense that there is irregular daily alternation between gross hyperglycemia and overt or occult hypoglycemia. In these patients, many factors are involved, such as poor diet, irregular habits, subcutaneous reactions at the sites of insulin injection, insulin overdosage, inability to break down complex insulin, and infection—to name only a few. As a rule, tolbutamide and carbutamide are ineffective in such patients, although an occasional excellent response has led to the hope that some other sulfonyleurea would be helpful in achieving significantly improved clinical control.

With the development of chlorpropamide, which was thought to be a more potent agent, it was felt that an adequate trial in the brittle, unstable, and poorly controlled diabetic was justified. Patients were selected from the Diabetic Clinic at Jefferson Davis Hospital and referred to a special clinic for oral therapy. The patients attending the diabetic clinic at this hospital have been characterized in detail in the literature.⁴ In general they are in poor financial circumstances, of low intelligence, and poorly educated. Most are obese, and urinary tract infection is a major problem.

Chlorpropamide was started in large doses (as much as 1.5 gm. per day) in the poorly controlled diabetic receiving large doses of insulin. In general, the patient's insulin dosage was reduced by one half when chlorpropamide therapy was instituted. This was the usual practice if the patient was receiving more than 50 U. of insulin. Those with mild, stable diabetes received 500 to 1000 mg. per day as the initial dose, and insulin was discontinued. Treatment was carried out on an outpatient basis and the

* The work reported in this paper was supported by a grant from Chas. Pfizer & Co., Inc., Brooklyn, N. Y.

† Trainees in Diabetes, Training Grant Program, National Institute of Arthritis and Metabolic Diseases, Public Health Service, Bethesda, Md.

patients were provided with explicit instructions to communicate with one of the physicians on the metabolic service if anything unusual occurred. No additional instruction in dietetics was given to any of these patients; this was done deliberately to obviate the possibility that any degree of improved control would result from improved dietary habits. The patients were checked at weekly intervals until they had become stabilized on the oral therapy; the period between visits was then lengthened. Doses of insulin and chlorpropamide were altered as deemed advisable from the clinical course. White blood counts, differentials, blood urea nitrogens, thymol turbidity, blood sugar, and chlorpropamide blood levels were determined at each visit. The patients who missed appointments were asked to appear later. Sixty-one patients have received therapy for a time sufficient to allow evaluation.

Results

The status of the sixty-one patients is summarized in TABLE 1. Three patients died while receiving chlorpropamide therapy. One of these died

TABLE 1
STATUS OF PATIENTS OBSERVED IN STUDY

Deaths since chlorpropamide therapy instituted:		3
Not related to drug.....	2	
Related to drug.....	1	
Received therapy for 1 week for special metabolic study.....		3
Drug discontinued because of increasing B.U.N. & symptoms.....		4
Restarted on drug therapy.....	2	
Lost to follow-up.....		4
Uncooperative, drug therapy discontinued.....		6
Toxic reactions, drug discontinued.....		12
Withdrawn because of poor control.....		1
Receiving uninterrupted drug therapy.....		28
Total.....		61
Presently on therapy.....		30

of what appeared to be a myocardial infarction, following a typical allergic reaction to penicillin. Chlorpropamide therapy had been discontinued one week before death and could be connected in no way with the death. The second was a patient with advanced generalized arteriosclerosis who developed an extremely severe urinary-tract infection and died of septicemia. It was felt that chlorpropamide therapy was not involved in this patient's demise. The death of the third patient was felt to be related to the chlorpropamide therapy.

Case Reports

S. K. was a 61-year-old white female who was diagnosed as having mild diabetes mellitus in 1954. She had a marked aversion to injections and refused to take insulin. Control was good, but with minor stress she became

poorly controlled. She requested oral therapy and was started on 500 mg. of chlorpropamide daily in a divided dose. Approximately 2 weeks later she became ill and was treated by a private physician who made a diagnosis of respiratory tract infection. The patient was given Azotrex, which contains tetracycline, sulfamethizole, and Pyridium. The physician found no evidence of hepatitis at the time of his examination. Her symptoms became more acute, and 4 days later she was admitted to Jefferson Davis Hospital suffering with nausea, vomiting, dehydration, and jaundice. She rapidly developed electrolyte disturbances and congestive heart failure, and expired. At autopsy, there was hepatitis of the cholestatic type, most commonly seen with chlorpropamide idiosyncrasy. This case will be reported in detail elsewhere. It is felt that had this patient reported to the clinic at the time she became ill, as she had been instructed, she probably would have survived, since she continued on sulfonylurea therapy from the onset of symptoms until admission to the hospital.

Significant improvement in controls was achieved in four patients, but the drug was discontinued because of an increasing level of blood urea nitrogen accompanied by symptoms of malaise, weakness, and lethargy. It was originally felt that this might be a consequence of drug therapy, but it is now felt that an acute flare-up of chronic urinary tract infection caused most of the symptoms. Two of the four patients have received chlorpropamide therapy again with no untoward effect. Six patients were lost to follow-up.

Patients who had been actively uncooperative in the therapy preceding that with chlorpropamide often continued this erratic behavior after being started on oral therapy. It is our impression that these patients did not do well, especially when judged by a sense of well being, general appearance, and clinical course. Often their chemical control was quite good. Chlorpropamide blood levels fluctuated quite widely, probably indicating that they had taken their tablets erratically. It is felt that patients who continue uncooperative and erratic should not be continued on this form of therapy. Toxic reactions of such severity as to force abandonment of chlorpropamide therapy occurred in 12 patients, 2 showing somnolence, 4 dermatitis, and 6 gastrointestinal symptoms.

It was necessary for the families of 2 patients with somnolence to go to extremes to awaken them to receive food and medicine. One patient, who developed a skin rash, also experienced mild to moderate somnolence on the drug. Of the 12 patients who discontinued chlorpropamide because of toxic reaction, 6 had received a maximum dose of 1000 to 1500 mg. daily and 6 had received a maximum of 250 to 750 mg. daily. Of the 32 who had discontinued drug therapy at some time, 10 had received at least 1500 mg. of chlorpropamide for days to weeks while, of the 30 presently on therapy, 13 have received 1500 mg. or more daily for a week or longer. Hypoglycemia was not mentioned in the side effects as this is a natural consequence of the drug therapy; with the large doses being given in this study, hypoglycemia was a common phenomenon. A large percentage of all of the patients treated had mild hypoglycemia some time before they were stabilized.

Ten of the patients underwent moderate hypoglycemic episodes, while 3 had severe hypoglycemia.

Of the thirty patients still receiving chlorpropamide, seventeen are in excellent control, three are in good control, and ten are in fair control; all are significantly improved relative to their condition during previous therapy.

Of the original 61 patients, 33 may be classified as poorly controlled or brittle diabetics; 19 of these are presently receiving chlorpropamide. This agent has proved to be extremely useful in improving the control of the patient who had been uncontrolled on other regimens. Chlorpropamide was used in combination with insulin in 13 of these cases. In 5, however, insulin

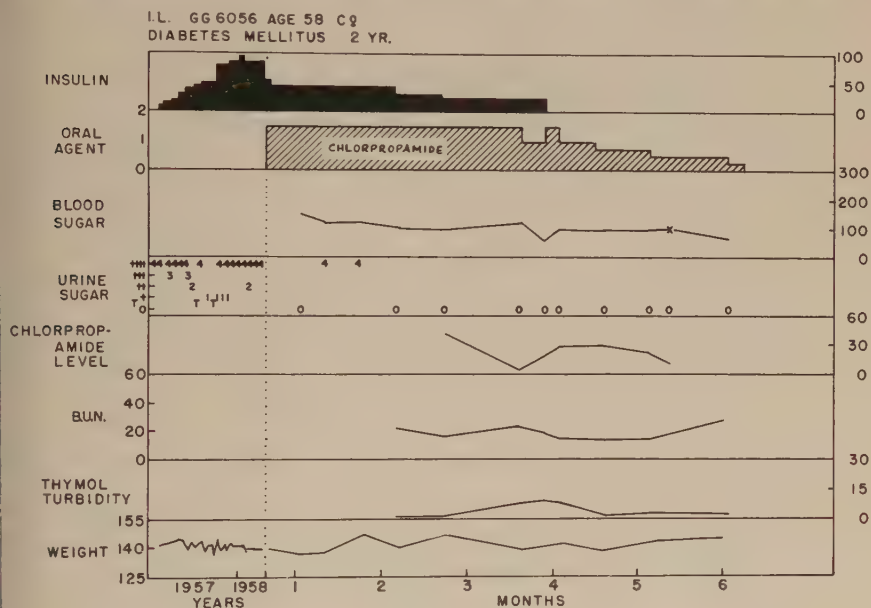


FIGURE 1

was eventually discontinued; chlorpropamide was used alone in patients who had received as much as 90 U. of insulin. The following case reports illustrate the results obtained.

I. L., a 58-year-old Negro female in whom the diagnosis of diabetes mellitus was made in 1956, was admitted to the hospital in September 1956 with a subacute myocardial infarction and congestive heart failure. She was treated with digitalis, diuretics, bed rest, and diet, with good results. Her course since that time is shown in FIGURE 1. She developed an almost constant 4-plus glycosuria associated with general lethargy and malaise and with occasional episodes of overt hypoglycemia. The response to chlorpropamide has been quite good. Insulin has been discontinued and the patient is maintained on 250 mg. of chlorpropamide daily.

B. S. is a 63-year-old Negro female with diabetes mellitus of 22 years' duration. When first seen at the hospital in 1939 she had a urinary tract

B S. GG 3069 63 YRS. C♀
DIABETES MELLITUS 22 YR.

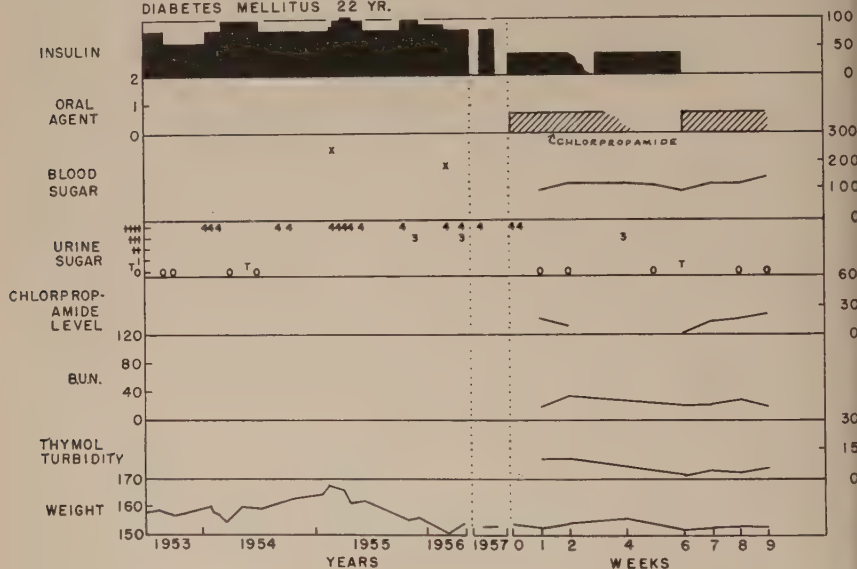


FIGURE 2

L. T., a 28-year-old white female with juvenile diabetes of 14 years' duration, was first seen at Jefferson Davis Hospital in 1951 with uncontrolled diabetes mellitus, mild acidosis, and pyelonephritis. She was admitted

1 month later with a carbuncle on the right buttock, acidosis, and severe pyelonephritis. In the period 1952 to 1956 she was admitted 5 times with severe urinary tract infection, usually associated with acidosis. It was possible to suppress the urinary tract infection, but not to eradicate it, in spite of all diagnostic and therapeutic procedures. In 1957 she was found to have a peripheral neuropathy, and Kimmelsteil-Wilson syndrome was suspected for the first time. Just after starting chlorpropamide therapy she again developed severe urinary tract infection accompanied by pneumaturia, with *Escherichia coli* the offending organism. This proved to be resistant to all forms of antibiotic therapy. In spite of the infection, it was possible

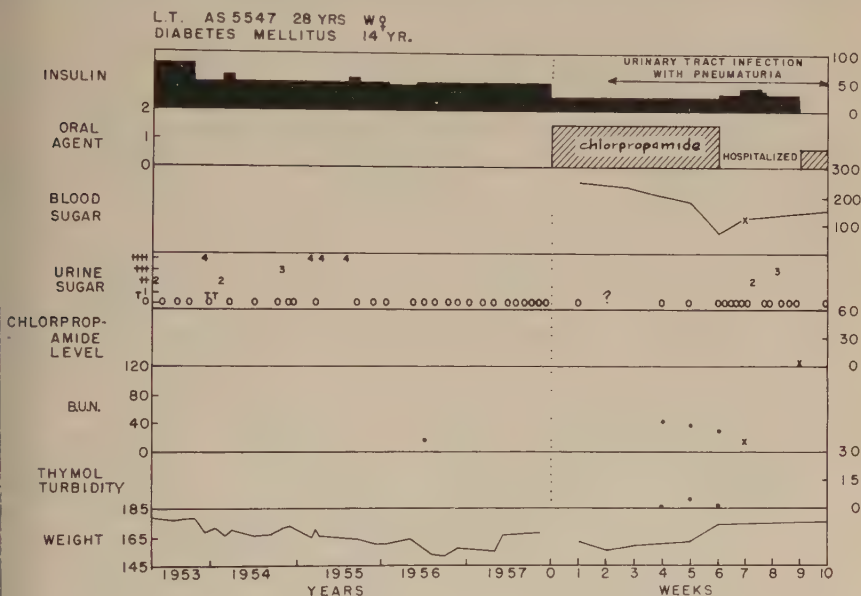


FIGURE 3

to discontinue the use of insulin and to achieve adequate control with 750 mg. of chlorpropamide daily. Dissatisfied because of the continued urinary tract infection, she did not cooperate with the oral hypoglycemic therapy and began to manifest evidence of poor control. This patient is classified in our uncooperative group of patients. It was evident that, as long as she cooperated with therapy, her control (both chemical and symptomatic) remained quite good; when she became uncooperative she rapidly developed symptoms that required discontinuation of chlorpropamide and reinstitution of insulin. This patient's history is summarized in FIGURE 3.

S. W., a 30-year-old Negro male in whom the diagnosis of diabetes mellitus had been made four years previously, had been hospitalized for two weeks in January 1955 with poor control and acidosis. He had previously failed to respond to carbutamide and tolbutamide. All general manifestations of poor control were present: such symptoms as weight loss, weakness, inability

to carry out sustained effort, and increasing susceptibility to infections. The response to chlorpropamide in this patient has been excellent. As in this patient, it is easy to see that chlorpropamide is effective when others of the sulfonylurea compounds have proved ineffective. This history is shown in FIGURE 4.

P. D., a 55-year-old white female with known diabetes of two years' duration was first seen at the hospital in May 1955 with paroxysmal auricular tachycardia that responded to parenteral digitalis. She was hospitalized for four weeks in December 1956 because of poor diabetic control, angina pectoris, and a posterior myocardial infarction of indeterminate age. During

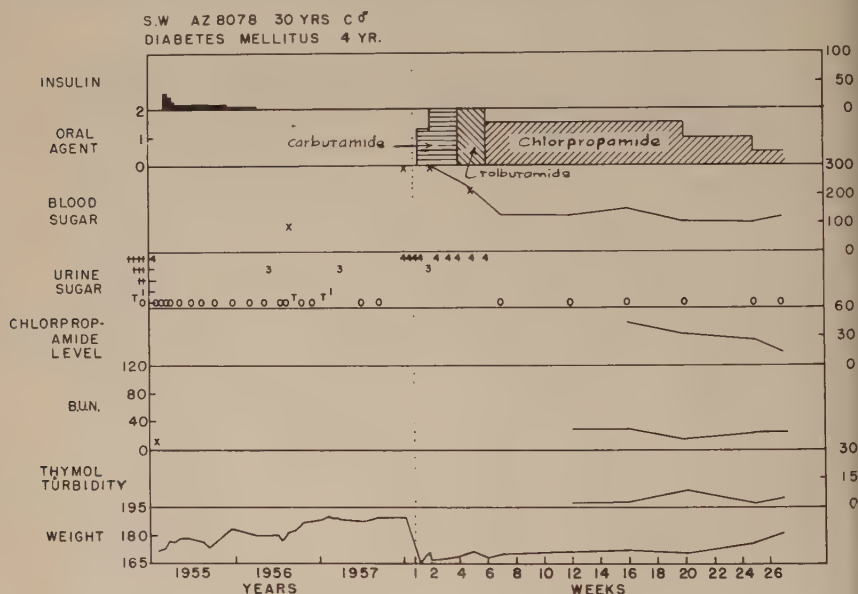


FIGURE 4

this hospitalization, tolbutamide therapy was started but was discontinued with the onset of an acute severe urinary tract infection. Later she was treated with carbutamide with fair initial response but secondary failure. Initial insulin therapy produced almost no improvement. When the insulin dosage was increased the patient developed rather severe hypoglycemia at irregular intervals. She developed severe hypoglycemia on the first day of combined therapy of chlorpropamide and insulin. Reduction in the chlorpropamide dosage led to significantly improved control. This patient subsequently left the community, and recent communication with her private physician at her new location gave the information that she has remained in excellent control. Data on this history are in FIGURE 5.

A. N. is a 59-year-old white female whose primary difficulty has been extensive nodule formation at the sites of insulin injection. As a consequence, she has alternating periods of hyper- and hypoglycemia that, at

times, have completely disrupted her life. With chlorpropamide therapy, the dose of insulin given has been reduced significantly; the nodule formation has not been a major problem at this reduced dose level of insulin. Details of this history are shown in FIGURE 6.

B. C. is a 66-year-old white female with diabetes of thirteen years' duration and has been observed for more than twenty years at the Jefferson Davis Hospital. This patient has myxedema and has had surgical removal of a nontoxic benign thyroid adenoma. For several years she has had recurrent acute episodes of a chronic severe urinary tract infection. She has arteriosclerotic heart disease with a left bundle branch block and peripheral neu-

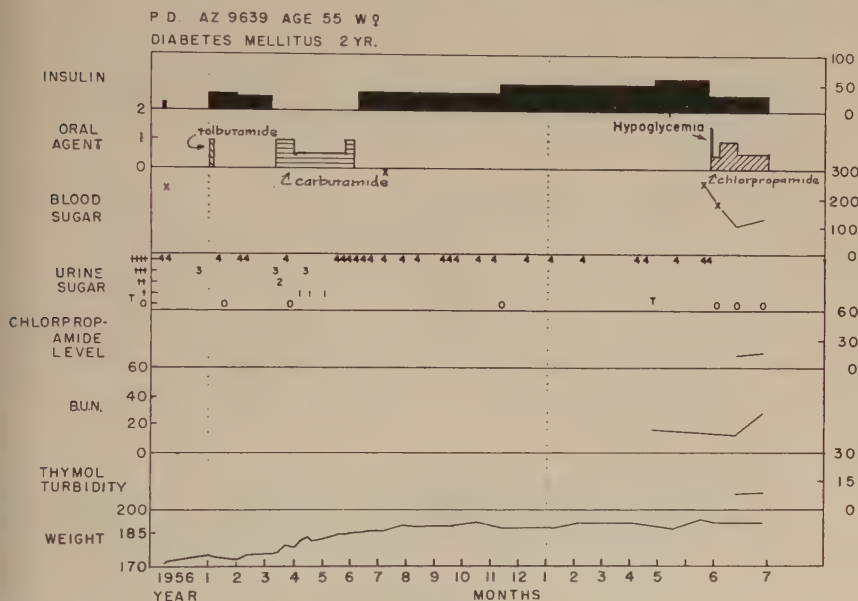


FIGURE 5

ropathy. At no time has this patient's diabetes come under satisfactory chemical control, in spite of all efforts in the Outpatient Clinic, plus multiple hospitalizations. The response to chlorpropamide has been dramatic; however, she continues with urinary tract infections, and the weight loss noted in the chart is due to the continued infection rather than the chlorpropamide therapy. This case is illustrated in FIGURE 7.

L. W., a 70-year-old white male with diabetes mellitus of 20 years' duration, was admitted to Jefferson Davis Hospital in 1950 with acidosis, and in 1953 with the same complaint. In 1957 he was found to have arteriosclerotic heart disease with congestive heart failure, which responded to digitalis, diuretics, and salt restriction. At the time of this hospitalization he also had mild hypertension and bronchopneumonia. He has received as much as 130 U. of insulin daily, with a clinical course characterized by alternating hyper- and hypoglycemia. He refuses to take less than 100 U.

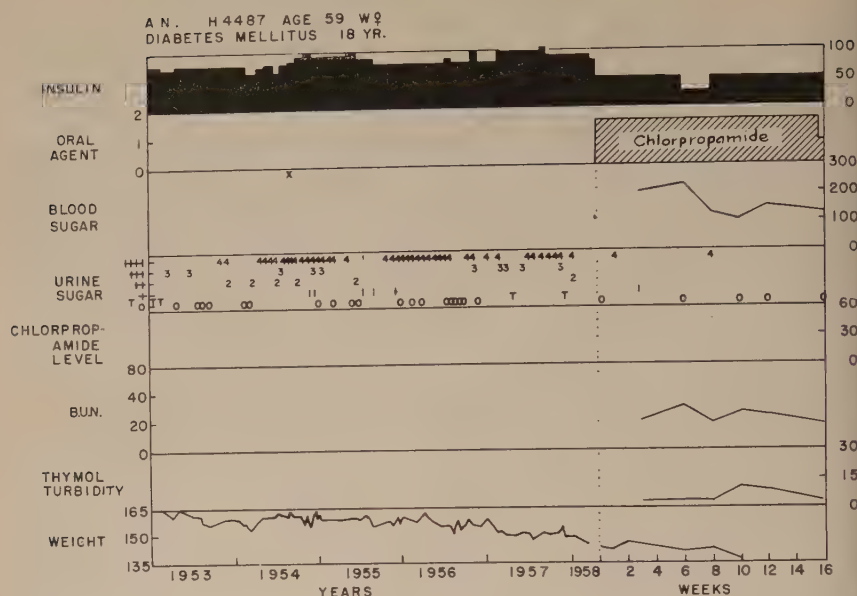


FIGURE 6

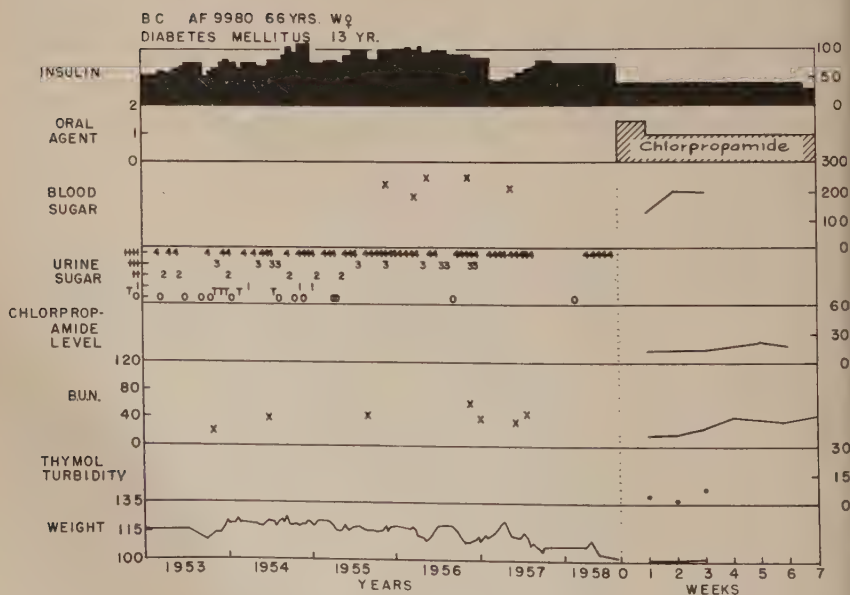


FIGURE 7

of insulin a day; however, the addition of chlorpropamide to 1500 mg. daily has significantly improved his chemical control. His clinical course is much smoother as he does not alternate between hyper- and hypoglycemia and feels much better than he has for many years. Details are shown in FIGURE 8.

J. J. is a 35-year-old Negro female with diabetes mellitus of 7 years' duration. At onset her diabetes was relatively mild and comparatively easily controlled. In the past two years, however, she has presented one of the most unusual and bizarre problems in diabetic control that has ever been seen in this clinic. She has developed extensive nodule formation at the sites of insulin injections and, in addition, approximately once a month,

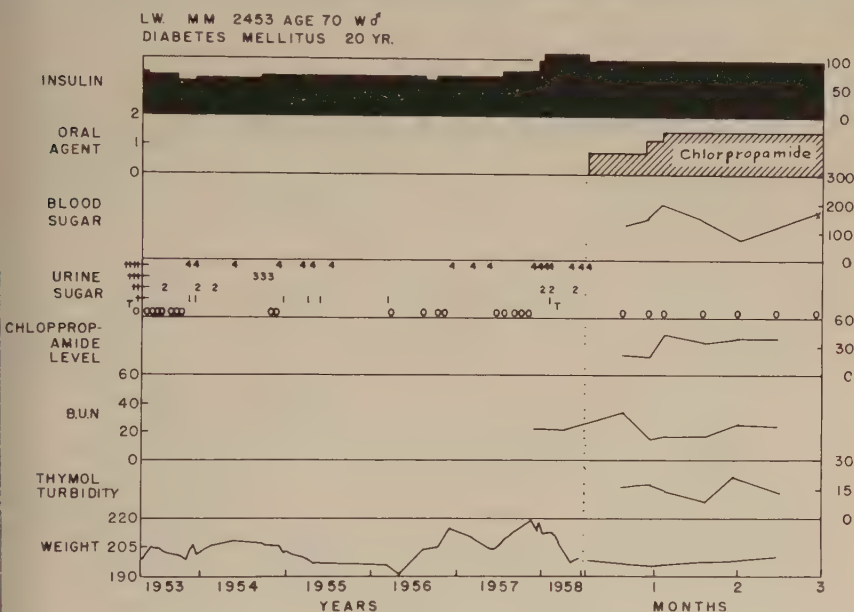


FIGURE 8

develops a delayed hypersensitivity response at the sites of injection. During these two years it has never been possible to achieve significantly improved control in spite of the fact that it has been possible to put her into an environment that is well controlled, where she receives meals regularly, and where nutritional deficiencies are corrected. She was first started on DBI. By chemical criteria this produced only minimal improvement in control; by clinical criteria, however, the results were quite striking. It was then felt desirable to change from DBI to chlorpropamide to determine if any improvement in the chemical control could be achieved. As may be seen in FIGURE 9, there was very little difference in the control achieved by each of these drugs. It should be noted, however, that she is receiving about 50 per cent of her previous dose of insulin. It is obvious, too, that occasionally she exhausts her insulin supply and neglects to return to the hospital for an additional supply. As a criterion for the improvement in control in this patient it

may be stated that in the $1\frac{1}{2}$ years prior to the institution of oral therapy, this patient had been hospitalized one fourth of the time. Since the institution of oral therapy she has not been hospitalized.

W. A. is a 64-year-old white male with diabetes mellitus of three years' duration. When the diagnosis was first established, he was treated with small doses of insulin. However, he failed to return to the clinic and was not seen again until early in 1958.

Because of the previous good results of chlorpropamide it had been the desire of the metabolic section to attempt to treat a person in acidosis with intravenous chlorpropamide. On admission, this patient was in moderate

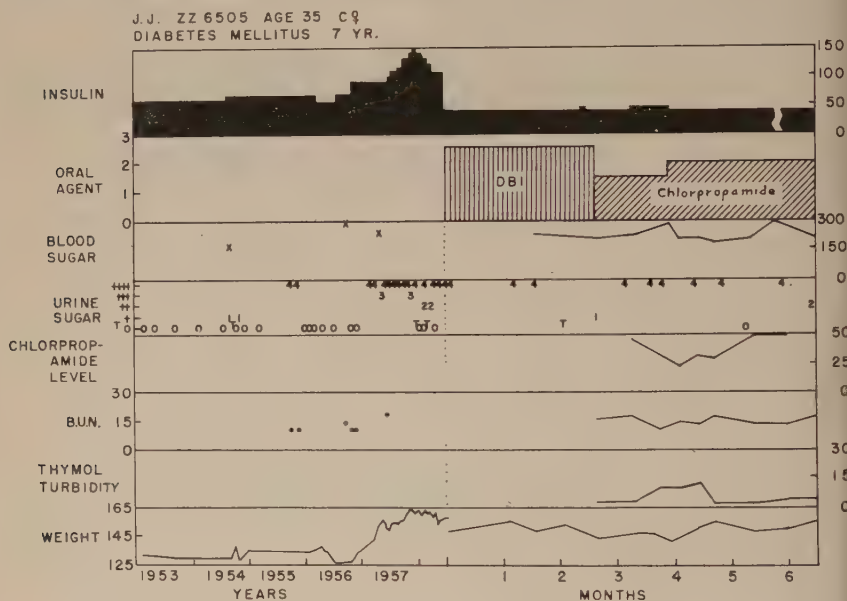


FIGURE 9

acidosis with clinical signs and symptoms, but he was not in coma. He was administered 1000 mg. of chlorpropamide intravenously during a 6-hour period. Other fluids were given as is indicated in FIGURE 10. It is evident that the patient's acidosis was relieved promptly. Glycosuria and hyperglycemia continued under the impact of the intravenous glucose. A total of 70 U. of insulin was given after the patient was cleared of acetonuria, more in an attempt to decrease the glycosuria than to control the acidosis. It is generally realized that mild to moderate acidosis may be treated with intravenous fluids and electrolyte replacement, without the administration of insulin. However, this result in 1 patient indicates that the use of chlorpropamide in diabetic acidosis may be worth further exploration.

As may be seen, practically all the patients received large doses of chlorpropamide for weeks to months. They tolerated these large doses quite well, and it is our impression that the only toxic manifestations that occurs

receiving oral therapy, and only one is receiving this in combination with insulin.

Summary

Chlorpropamide has proved to be a valuable adjunct to the therapy of brittle and poorly controlled diabetics. In patients receiving large doses of insulin it has been possible in most instances to reduce the dose of insulin by at least 50 per cent and to achieve a significantly smoother and more stable course for the patient. One case of toxic hepatitis with death is reported; apparently this was due to idiosyncrasy to the drug. Chlorpropamide remains effective under such stress situations as infection and surgical operation and, on the basis of one case, in the presence of acidosis.

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Discussion of the Paper

GARFIELD G. DUNCAN (*Philadelphia, Pa.*): I have three patients who became refractory to tolbutamide therapy and later required a larger dose of insulin than previously. Have any of the investigators in basic research repeated the experiments of Allen, who performed a partial pancreatectomy in dogs to produce a state just short of diabetes and then provoked diabetes by overfeeding, thus stimulating the islets of Langerhans?

It seems to me that there may exist a similarity between these experiments and experiences with the patients who require more insulin than before sulfonylurea treatment. I suggest that we do the same thing and try to precipitate diabetes in such partially pancreatectomized animals with the sulfonylurea compounds. Although this may have been done, I have not heard of it.

MARTIN G. GOLDNER (*Jewish Chronic Disease Hospital, Brooklyn, N. Y.*): The question whether secondary failures to sulfonylureas have an increased insulin requirement is an important one. From what I have seen and from what I know of the literature, Lamar's experience does not appear to be the rule. I recall, for instance, Pfeiffer's¹ series of thirty secondary failures; only three or four of these had an increased insulin requirement, and the increase was rather small, while all others required the same amount or even less than that needed prior to sulfonylurea treatment. However, one must realize that there are at least three different types of so-called secondary sulfonylurea failures: there is, first, the patient who overeats and relaxes his diet when placed on the drug; second, the true secondary failure who, without any detectable cause, escapes from control; and, third, the patient in whom the diabetes itself has become worse. We must not forget that we deal with a disease that may be stable for some time, but that may also progress at any

time. It would not be surprising if the first or the third type need more insulin, but neither would indicate true failure of the sulfonylureas. The first is a failure of dietary management, the third an idiopathic aggravation of the disease. Only the second group can be linked causally to the hypoglycemic drugs, and in this type the general experience seems to be that the insulin requirement is practically unchanged.

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ADVANTAGES AND DISADVANTAGES IN SHIFTING PATIENTS FROM TOLBUTAMIDE TO CHLORPROPAMIDE

J. Blöch and A. Lenhardt

Metabolic Disturbances Service, Krankenhaus der Stadt Wien-Lainz, Vienna, Austria

Because of the chronicity of diabetes mellitus, permanent continuous treatment is necessary. Therefore, efforts have been made (and continue to be made) to develop new clinically effective agents that have pronounced hypoglycemic effect. It is most desirable that minimal dosage of such agents produce an optimal antidiabetic effect and, further, that the lowest possible incidence of untoward side reactions be associated with therapy. In keeping with this research objective, the Austrian branch of Pfizer International, Inc., New York, N. Y., supplied us with the new antidiabetic drug, chlorpropamide, for clinical evaluation.

Materials and Methods

Two hundred inpatients were treated with chlorpropamide, special consideration being given to evaluating the clinical advantages and disadvantages of the drug in comparison with those of tolbutamide and carbutamide.

TABLE 1
DATA ON DIABETICS SUCCESSFULLY CONTROLLED WITH CHLORPROPAMIDE

Previous measures	No. of patients	No. with good control on chlorpropamide only
Diet only.....	45	43
Tolbutamide and carbutamide.....	26	20
Insulin and introductory chlorpropamide.....	56	40
Prolonged insulin.....	73	43
Total.....	200	146

More than 1700 diabetic patients have been treated with the latter 2 preparations for a period of almost 3 years. In addition, 15 outpatients were put on chlorpropamide during our clinical investigation.

In July 1958 we were able to report on 136 patients studied to that time. We have now enlarged this group to 200 patients who either had been on insulin or whose diabetes had not been satisfactorily controlled by dietary restriction alone, even though they were hospitalized. The above figure also includes those patients who had been treated with tolbutamide, carbutamide, or both. TABLE 1 shows that 146 patients (more than two thirds of the diabetics who appeared suited to oral therapy) could be well controlled with chlorpropamide. Of the 146 patients, 63 had not been controlled by insulin; the remaining 83 had been. Of the latter, 40 received insulin only on

admission to the hospital in order to stabilize their diabetic condition rapidly; the remaining 43 had been controlled with insulin for more than 10 years.

The protracted therapy employed for periods as long as 5 months (follow-ups were carried out at 3-week intervals) brought about very good metabolic equilibrium in 25 of 38 cases, and just sufficient control in 5 cases. In the latter, fasting blood sugar was about 200 mg. per cent, and 24-hour glycosurias were up to 15 gm. In the remaining 8 patients, control with chlorpropamide was discontinued because of increasing glycosuria, which obviously

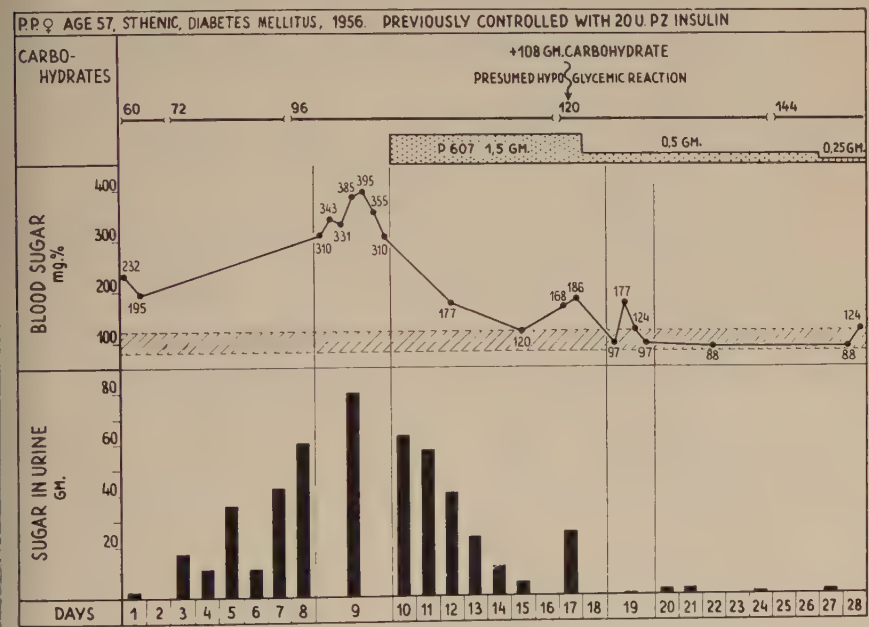


FIGURE 1. Chlorpropamide-induced increase in carbohydrate tolerance.

resulted from neglect of the strict dietary rules. Acidotic and pregnant diabetics were not treated with chlorpropamide. The usual complications, for example retinopathy, sclerosis of the coronary arteries, nephropathy, and obliterating arteriosclerosis of the extremities do not constitute contraindications for chlorpropamide treatment. It follows that the control of diabetes with chlorpropamide is more successful—the majority of the patients respond—in those in whom diabetes becomes manifest at or after the fifth decade of life. This applies especially to diabetics who previously were controlled by dietary restriction alone (TABLE 1). In such cases chlorpropamide allows a larger intake of carbohydrates while effecting a nearly normal fasting blood sugar, an excellent 24-hour blood sugar profile, and sugar-free urine (FIGURE 1).

Also evident is the fact that, in patients taking insulin, the possibility of success with chlorpropamide decreased as the need for insulin increased. In the majority of cases requiring less than 40 U. insulin daily, chlorpropamide

could be substituted with success. However, even in 21 diabetic patients requiring as much as 60 U. of insulin daily, we succeeded in achieving good metabolic equilibrium with chlorpropamide. Regarding duration of the disease and, to a lesser extent, duration of insulin medication, quite favorable

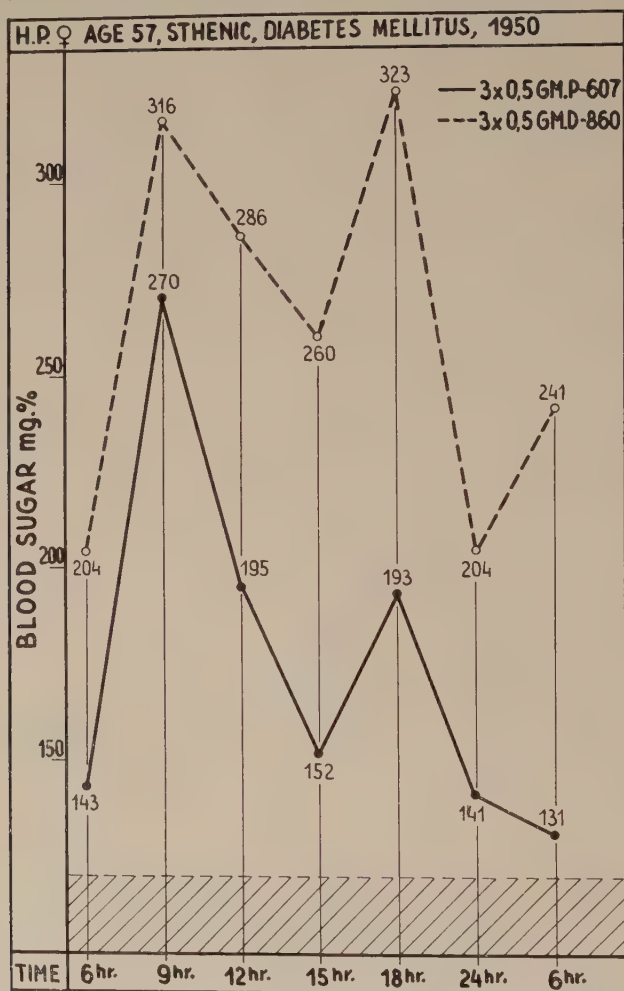


FIGURE 2

exceptions to the 10- and 5-year rule, respectively, could be obtained with chlorpropamide, as was demonstrated in 26 diabetics in whom we replaced insulin completely, although they had been receiving insulin for 10 to 20 years. Recent diabetics, even those of the asthenic type, sometimes did well on chlorpropamide if they were well beyond 40 years of age.

Regarding the responses in the two large diabetic groups, the brittle and the stable, it may be stated that, in general, the same criteria are applicable

with chlorpropamide as with the oral antidiabetic drugs previously used. However, as with other drugs, exceptions exist and, because mixed forms result from the overlapping of the classifications brittle and stable, the exceptions increase.

Observations

The effect of chlorpropamide in reducing blood sugar and sugar in urine is greater than that of the two other oral antidiabetics now in use. Hence,

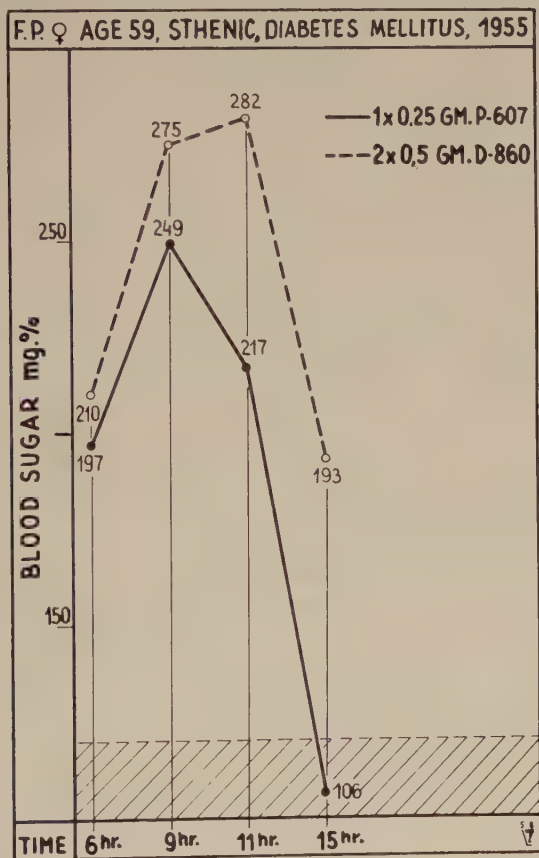


FIGURE 3

with a lower dosage of chlorpropamide (sometimes substantially lower, especially during the initial treatment period), equally favorable therapeutic results can be achieved together with good equilibrium of metabolism (FIGURES 2, 3, 4, and 5). The initial daily dosage usually was 1 gm. (two 500-mg. doses) and, in some cases 1.5 gm. (three 500-mg. doses). Maintenance dosage was usually 0.5 gm. or 0.25 gm., frequently given every other day. In patients on relatively low quantities of carbohydrates (for example, 80 to 120 gm. daily) higher initial dosage can lead to sudden hypoglycemic episodes,

especially if the treatment is preceded by a considerably restricted, although short-term, diet (48 to 60 gm. carbohydrate).

The following episode supports the theory (of the mechanism of action of the oral antidiabetic drugs) that these preparations promote or activate endogenously produced insulin. The dextrose loading test carried out in a nondiabetic (FIGURE 6) who, because of obesity, was put on a restricted diet (especially with respect to carbohydrates) showed very good endogenous

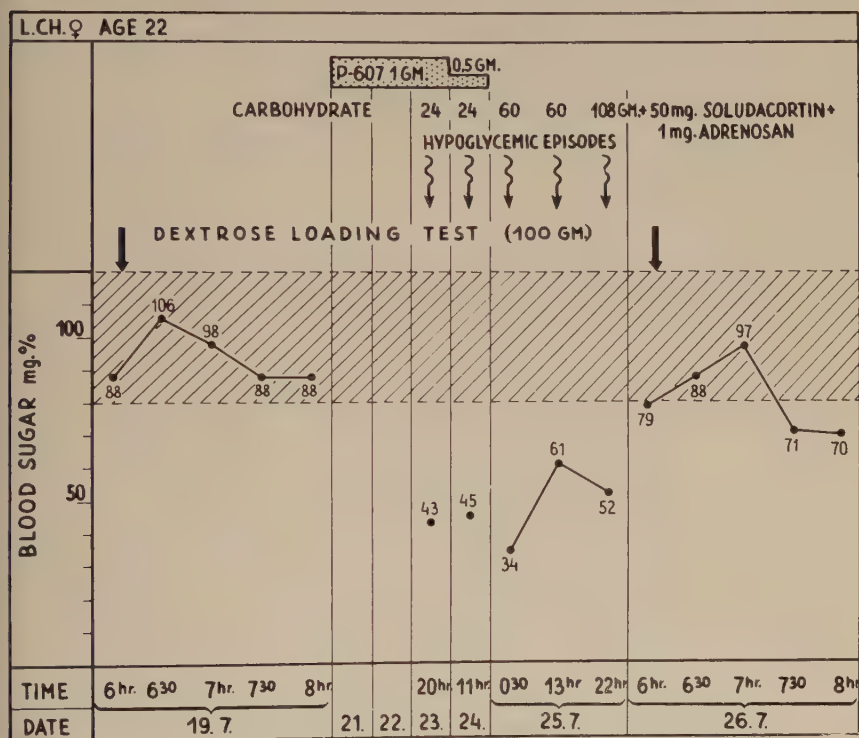


FIGURE 6. Promotion by chlorpropamide of endogenously produced insulin.

insulin action. After the administration of six 500-mg. doses of chlorpropamide in a period of 3 days, the patient suddenly experienced a serious convulsive hypoglycemic episode accompanied by unconsciousness and a decrease in the blood sugar level to 43 mg. per cent. During the next day the patient remained in a serious hypoglycemic condition, blood sugar levels varying between highs of 34 and 61 mg. per cent. The hypoglycemia could be combated only by continuous glucose administration (260 gm.), part being given intravenously. We also administered injections of Soludacortin and adrenalin. A repeat of the loading test, done with 100 gm. of dextrose and performed on the third day, brought about blood sugar levels of 70 to 88 mg. per cent, with one peak of 97 mg. per cent.

Because of this markedly greater hypoglycemic action, it would be possible

to treat with smaller doses of chlorpropamide diabetics who require far more exogenous insulin; this would not generally be the case with tolbutamide. This action, however, may be regarded as an advantage only if prolonged use and careful laboratory testing show that the chronic-toxic effects of the drug or its degradation products have no deleterious effects, particularly on the liver, kidneys, and the hematopoietic systems. The problem of toxicity in this regard is of special interest because we may assume that the ability of chlorpropamide to reduce blood sugar is due to its considerably delayed excretion, and that the prolonged effectiveness of the lower dosages is due primarily to cumulative action.

In view of the above evidence and its substantiation by clinical observation, we can state that the initial adjustment period for chlorpropamide is longer (as much as one week) and the duration of action more prolonged than with tolbutamide.

Of 215 patients (including outpatients) untoward side reactions were observed more often at the beginning of the treatment and less frequently after therapy had been in progress for some time. Gastrointestinal disturbances were noted in 10.7 per cent of cases, dermatological side reactions in 3.5 per cent, and intolerance to alcohol in 11.6 per cent.

Gastrointestinal disturbances occurred in twenty-three cases and manifested themselves as loss of appetite (often to a great degree), malaise, and occasional nausea and vomiting. Sometimes these disturbances were associated with other complaints in the upper abdomen (for example, pressure on the stomach, gastric cramps, and bloating) and, although in only a few cases, with diffuse abdominal colic and constipation. Incidence of these side reactions lowered dramatically when the initial dosage or maintenance dosages were reduced substantially. This, however, necessitated a prolonged initial period of adjustment. Thus, the disturbances proved to be dependent on dosage and subject to considerable individual fluctuation.

Toxic and allergic skin reactions such as urticaria, edemas, and generalized morbilliform exanthemas were observed in 7 patients while transitory itching of the skin was noted in 4. The latter symptom never became so marked that it necessitated discontinuation of the preparation, and it subsided without requiring therapy. In 2 cases of morbilliform exanthemas, we were able to continue medication under antiallergic protection. Marked changes in the differential blood count of the white blood corpuscles (for example, the eosinophils) were not observed in association with the above skin reactions.

As to the hematopoietic system, no toxic effects were noted except for occasional granulocyte decrease which, as proved by periodic follow-ups, was only transitory. No hepatic damage or adverse effect on the kidneys or the central nervous system became manifest during the time of observation, which was as long as five months.

Flushing, subjectively often felt as "congestion" and a warmth of the face and neck, visible as a temporary redness sometimes extended to the upper thoracic area. It was observed in 31 patients (regularly in 24) after consumption of even a small quantity of alcohol. The above-mentioned symptoms generally occurred 10 to 15 min. after taking a small quantity of alcohol,

regardless of the type of alcohol imbibed, and were similar to an Antabuse-alcohol reaction. Of course, these side reactions may be prevented by avoidance of alcohol.

A few patients complained of giddiness and buzzing in the ears; these symptoms, however, cannot be clearly designated as side reactions, because their interpretation allows so many other possibilities to be taken into consideration.

Conclusion

It may be stated that chlorpropamide has two advantages over the other antidiabetic sulfonylurea preparations: first, minimal dosages will produce an entirely satisfactory hypoglycemic effect that compares favorably with that of the other antidiabetic drugs previously used; and second, it is distinguished by an especially prolonged duration of hypoglycemic action, so that in no case need it be given more often than once a day and it may be given as infrequently as every other day. A higher incidence of dyspeptic disturbances must be looked upon as a disadvantage; however, these disturbances were observed principally during initiation of therapy, when relatively high doses were being given.

CHLORPROPAMIDE IN THE MANAGEMENT OF THE ADULT DIABETIC PATIENT*

Norman W. Drey, Barrett L. Taussig, Jim Waterfield, Robert Rubin
Medical Section, The Research Institute of The Jewish Hospital of St. Louis, St. Louis, Mo.

This article will describe our experiences with chlorpropamide in the clinical management of adult diabetic patients.

Methods and Results

Our observations were made on 32 diabetic patients who received chlorpropamide for as long as 36 weeks (FIGURE 1). The study included 20 females and 12 males, with an age range of 33 to 74; 26 were in the range of 50 to 70

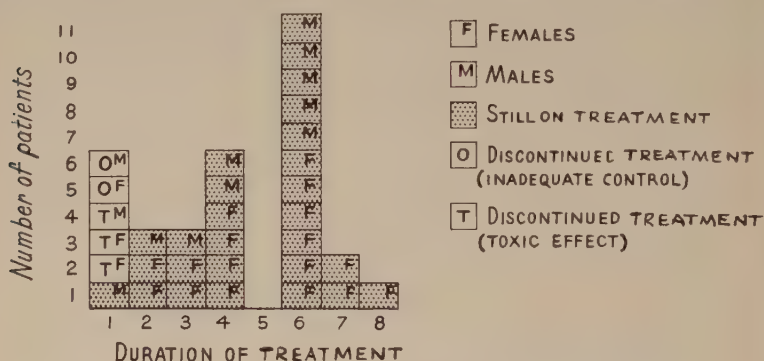


FIGURE 1. Duration of chlorpropamide therapy in 32 patients.

(TABLE 1). Constant dietary regimen has been maintained, most of the patients having 2000-cal. diets consisting of 250 gm. carbohydrate, 90 gm. protein, and 70 gm. fat—divided into meals of approximately equal caloric value. This basic diet plan has been changed in a few cases where body weight indicated need for adjustment. Thirteen patients were observed initially in the hospital and 19 have been handled entirely as outpatients. Diabetic control has been checked by frequent fasting and 2-hour postbreakfast plasma glucose analyses¹ and frequent determinations of 24-hour glycosuria.²

A systematic study of possible toxicity has been carried out and has included frequent clinical observation of the patient. There have been periodic determinations of body weight, serum cholesterol, BSP retention, total and fractional serum proteins, cephalin-cholesterol flocculation, serum alkaline phosphatase, icterus index, blood urea nitrogen, urine analysis, and platelet and complete blood counts. There have been occasional prothrombin time determinations. Serum diastase, transaminase, and bili-

* The work on which this paper is based was supported by a grant from Chas. Pfizer & Co., Brooklyn, N. Y., who supplied the chlorpropamide used in the study.

rubin levels have been investigated in a few of the cases. Needle biopsies of the liver also have been obtained in a few cases.

In general, our earlier cases received initial doses of 1.5 to 1.0 gm. The frequency of mild hypoglycemic reactions caused us to decrease the initial dosage to 0.5 gm. or less by the time we had had experience with the first 15 patients (TABLE 2). Only 1 patient received an initial dose of more than 1.0 gm. Chlorpropamide was discontinued in 2 patients because of inadequate control; both had had good control on insulin. In one, chlorpropamide was started with progressive decreases in insulin, but the improvement was

TABLE 1
AGE DISTRIBUTION AND STATE OF CONTROL OF 32 PATIENTS WITH DIABETES MELLITUS

Age range	No. of cases	Control on chlorpropamide	
		Adequate	Inadequate
30-40	1	1	0
40-50	4	4	0
50-60	11	10	1
60-70	15	14	1
70-80	1	0	1
Total.	32	29	3

transient, as demonstrated by increasing glycosuria and insulin requirements. In the other case, brought to the hospital in a state of insulin-produced hypoglycemia, an attempt made to control the patient with chlorpropamide alone was unsuccessful. Chlorpropamide was discontinued in 3 cases because of possible toxicity. In 2 others, chlorpropamide was discontinued temporarily and resumed without further difficulty when it was decided that accompanying skin reactions were unrelated to the drug.

Clinical Observations

In assessing control, the same criteria were used as in judging effectiveness of tolbutamide in the therapy of adult diabetes mellitus.^{3, 4} Adequate control was designated "good" or "fair." A patient was considered to have a good response if one of the following phenomena was observed: a disappearance of the insulin requirement in the insulin-treated patients; a disappearance of glycosuria with an associated decline in plasma glucose levels in the noninsulin-treated, glycosuric patients; or a marked fall in the fasting and postprandial plasma glucose levels in the aglycosuric patients, when compared to similar levels without chlorpropamide. These biochemical aspects of control were considered together with the following clinical objectives: clinical well-being at all times (that is, freedom from any symptoms); achievement and maintenance of ideal body weight; absence of ketosis and hypoglycemic reactions. A given patient was considered to have a fair

TABLE 2
SUMMARY OF 32 ADULT DIABETIC PATIENTS TREATED WITH CHLORPROPAMIDE

Case No.	Sex	Age	Duration			Complications	Date of initial treatment	Date treatment discontinued	Initial dose (gm.)	Maintenance dose (gm.)	Response*
			Diabetes (years)	Insulin treatment	Tolbutamide treatment						
1	F	66	3	—	—	Paget's disease, cataracts.	11/30/57		1.0	0.1	G
2	F	57	0	17 days	—		1/7/58		0.5	0.1	G
3	F	51	10	2 ¹ / ₂ years	—	Retinopathy, mild.	1/16/58		1.0	0.5	F
4	F	33	0	0	0	Obesity.	1/20/58		1.5	0.5	F
5	M	61	20	10 years	2 weeks	ASCVD, retinopathy.	2/27/58	3, 20	1.0	1.0	G
6	M	70	19	—	2 months	ASCVD.	1/23/58	58	1.0	0.25	G
7	F	46	1	0	6 months		1/24/58		1.0	0.5	G
8	M	66	20	5 years	—	ASCVD, arteriosclerosis obliterans.	1/28/58		1.0	0.5	F
9	M	52	3	3 years	—	ASCVD, retinopathy.	2/6/58		1.0	0.25	G
10	M	68	18	20 years	6 months	ASCVD.	2/6/58		1.0	0.5	G
11	M	53	14	—	—	Retinopathy, neuropathy.	2/9/58		1.0	0.25	G
12	F	65	1	1 year	—	ASCVD, cataracts.	2/13/58		1.0	0.2	G
13	F	63	20	20 years	—	ASCVD.	2/13/58		1.0	0.5	F
14	F	45	—	—	—	Gallbladder disease, with stones.	2/24/58		1.0	0.1	G
15	F	62	22	16 years	—	ASCVD, hypertension.	2/20/58		0.5	0.5	F
16	F	68	5	5 years	—		4/3/58		0.5	0.1	G
17	F	46	—	—	—	Obesity, hypertension.	4/9/58	7/1/58	0.25	0.1	G
18	F	46	3	—	—	Retinopathy.	4/10/58		0.5	0.1	G
19	M	49	3	3 years	10 months		4/17/58		0.5	0.5	F
20	F	74	?	—	—	ASCVD.	4/17/58		0.5	0.5	F
21	F	59	2	—	—		4/17/58		0.1	0.3	G
22	M	58	—	—	—	ASCVD.	4/19/58		0.25	0.25	F

TABLE 2 (Continued)

Case No.	Sex	Age	Duration			Complications	Date of initial treatment	Date treatment discontinued	Initial dose (gm.)	Maintenance dose (gm.)	Response*
			Diabetes (years)	Insulin treatment	Tolbutamide treatment						
23	F	63	—	—	—	Obesity.	4/30/58		0.25	0.2	G
24	M	64	4	—	—	ASCVD.	5/12/58		0.1	0.1	G
25	F	59	8	—	—	ASCVD.	7/3/58	7/17/58	0.25	0.25	G
26	M	64	—	—	—	ASCVD, coronary disease.	5/29/58		0.2	0.2	G
27	F	55	—	—	—		6/12/58		0.25	0.5	G
28	F	58	5	—	1 year		6/17/58		0.5	0.5	G
29	M	59	26	26 years	—	Cataracts, osteoarthritis.	6/20/58	7/11/58	0.5	0.5	O
30	M	69	10	10 years	—		7/4/58		0.5	0.5	G
31	F	65	7	1 year	—	Hypertension, osteoarthritis.	7/11/58	7/31/58	0.5	0.5	G
32	F	67	22	22 years	—	ASCVD, neuropathy.	7/14/58	7/23/58	0.5	0.5	O

* Key: G, good; F, fair; O, inadequate.

TABLE 3
CONTROL ACHIEVED WITH CHLORPROPAMIDE THERAPY

Patients	Adequate		Inadequate	Total
	Good	Fair	Poor	
Number.....	22*	8	2	32
Percentage of total.....	68.7	25.0	6.3	100

* Includes three patients who showed good results for 2 to 3 weeks, but in whom treatment was discontinued because of toxicity.

response when the above criteria were partially fulfilled. TABLE 3 summarizes our experience. Thirty of our patients showed adequate control, 22 showed very good response, and 8 showed a fair or partial response. Two patients showed no response; both were diabetics of long standing (more than 20 years), with substantial insulin requirements.

This investigation has little statistical significance. However, observations in a few cases raised interesting questions. Case 11, M. P., is a 53-year-old male who had had mild diabetes for 14 years and who had had no previous therapy other than avoidance of "sweets" (FIGURE 2). Outpatient observations had established fasting plasma glucose levels at approximately 275 mg. per cent and 2-hour postbreakfast levels of more than 350 mg. per cent. His response to 1.0 gm. of chlorpropamide was immediate; the dose was

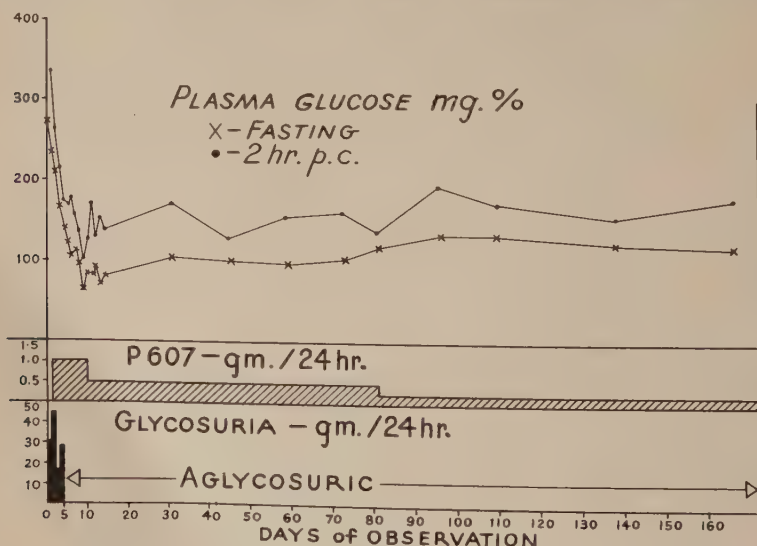


FIGURE 2. Case 11, M. P., male, 53 years old. Duration of diabetes, 14 years. No previous treatment.

reduced to 0.5 gm. on the tenth day because the plasma glucose level had fallen to 66 mg. per cent fasting, and 105 mg. per cent 2 hours after breakfast. On this dosage he was observed as an outpatient; plasma glucose levels remained in the range of 100 to 110 mg. per cent fasting, and 130 to 160 mg. per cent 2 hours after breakfast. Further reduction in chlorpropamide to 0.25 gm. once daily before breakfast has resulted in slight increases in the plasma glucose levels. Glycosuria disappeared on the third day of therapy and the patient has remained aglycosuric. The body weight has remained stable at 140 lb. (plus or minus 2 lb.) throughout the observation period.

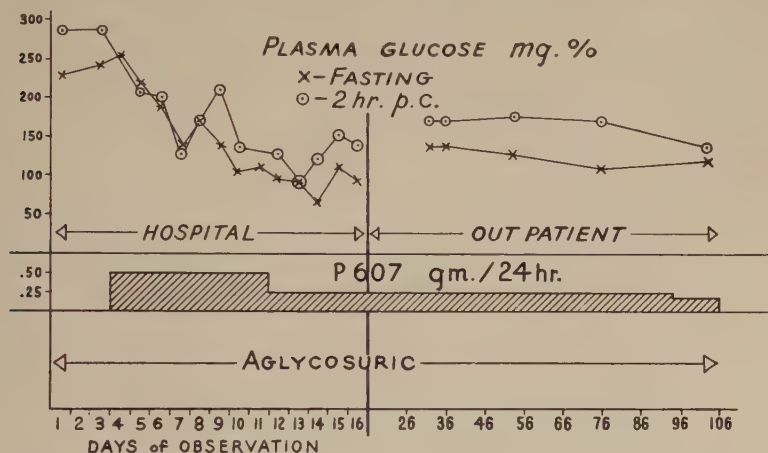


FIGURE 3. Case 18, I. R., female, 46 years old, diabetic 3 years. Previous treatment: diet.

Case 18, I. R., a 46-year-old female with diabetes of 3 years' duration and treated by diet alone, was aglycosuric throughout our observations (FIGURE 3). Her response in the plasma glucose levels to the initial dose of 0.5 gm. chlorpropamide was dramatic and compelled reduction to 0.25 gm. on the ninth day. There has been progressive weight loss from 173 to 163 lb., and the patient is well maintained currently on 0.2 gm. chlorpropamide daily.

Case 26, A. G., a 64-year-old male with newly discovered diabetes was handled entirely as an outpatient. His pretreatment fasting and 2-hour postprandial plasma glucose levels were of the magnitude shown in FIGURE 4 as the initial levels, that is, 250 mg. per cent and 319 mg. per cent, respectively. He has remained aglycosuric throughout, his chlorpropamide dosage has remained at 0.2 gm., and his plasma glucose levels have shown a very satisfactory response.

The postprandial plasma glucose level as an indicator of therapeutic effectiveness impressed us in evaluating tolbutamide therapy.^{3, 4} The three cases presented illustrate the same phenomena, and the longer-term observations support our impression that the prompt and substantial fall in the postprandial plasma glucose level bespeaks a good total response.

The information that approximately five days were required on a given

dosage of chlorpropamide to achieve a blood level plateau, and that the levels fell slowly in the two or three days following cessation of the drug, immediately suggested the possibility that we might observe in some of our cases a "chlorpropamide hang-over" effect in the plasma glucose levels.⁵ We believe we have observed this in one case.

Case 31, S. T., is a 65-year-old white female, diabetic for 7 years, who had been managed for the most part with diet alone, but who had also taken as much as 15 U. of insulin for short periods (FIGURE 5). Initially observed in the hospital, she seemed resistant to 0.5 gm. chlorpropamide given as a single daily dose before breakfast. After 6 days of this therapy, the fasting plasma glucose level was 189 mg. per cent and the 2-hour postbreakfast level was

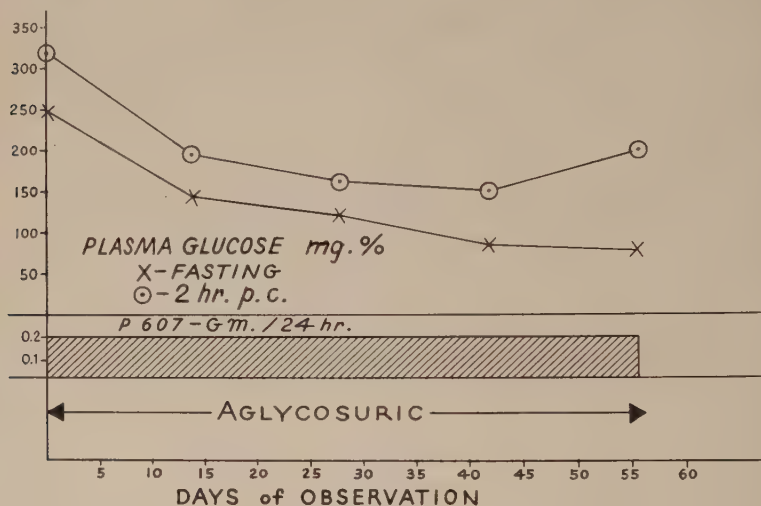


FIGURE 4. Case 26, A. G., male, 64 years old, new diabetic.

235 mg. per cent. She received placebo therapy the next 2 days; on the third day, plasma glucose levels were 145 mg. per cent and 246 mg. per cent, respectively. The relatively lower fasting plasma glucose level and the virtually unchanged 2-hour postprandial level after 2 days of placebo therapy suggested the possibility of a "hang-over" effect. The more favorable response when the drug was resumed remains unexplained. There have been no correlation studies between serum levels of chlorpropamide and plasma glucose levels. Observations in this case have led us to institute such studies. If the observation is validated, chlorpropamide may have a significant potential for successful intermittent therapy.

Observations Concerning Toxicity

With respect to toxicity, the last case, that of S. T., showed a cephalin-cholesterol flocculation reaction of three plus, nineteen days after instituting chlorpropamide therapy. The remainder of the liver profile, including serum transaminase levels, prothrombin time, and BSP retention, gave normal

results. In this case we were fortunate to have needle biopsies of the liver before starting chlorpropamide therapy, and these were repeated as soon as we had confirmed the three-plus cephalin-cholesterol flocculation reaction. No evidence of necrosis or any other change was detected with hematoxylin and eosin stain. One other case presented the same cephalin-cholesterol flocculation abnormality with no other abnormality in the liver profile. Needle biopsies of the liver were not obtained. In both cases the drug was stopped immediately.

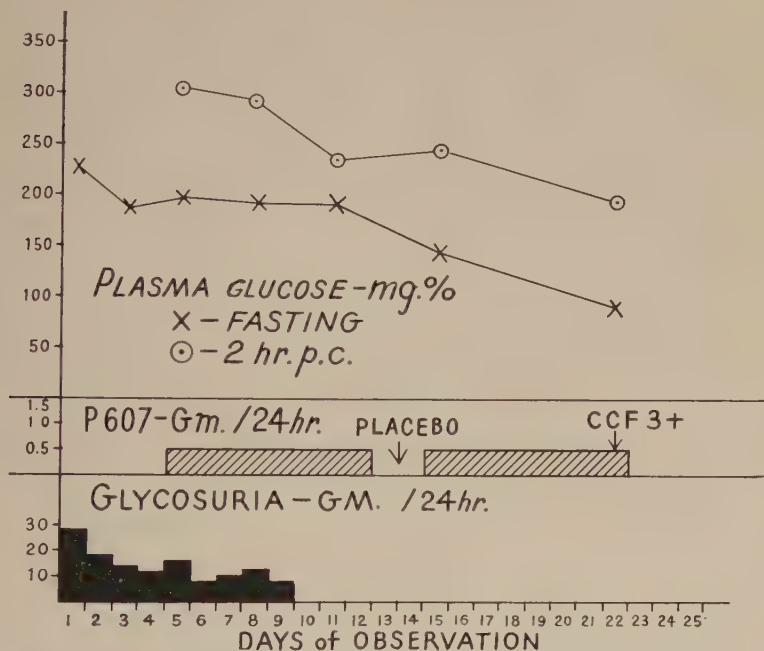


FIGURE 5. Case 31, S. T., female, 65 years old, diabetic 7 years.

In one other case the drug was stopped because the patient complained of nausea and a constant disagreeable metallic taste. This patient had done well previously on tolbutamide and, at his insistence, was returned to this therapy.

Summary

Thirty-two adult diabetic patients receiving chlorpropamide have been observed closely for periods as long as 36 weeks. Thirty of the patients showed adequate response, 22 showed a very good response, and 8 a fair response. Two of those showing a good response developed positive cephalin-cholesterol flocculation reactions after about 3 weeks of therapy, and the drug was discontinued. Two showed no response; both of these had had diabetes for more than 20 years and had had substantial insulin requirements. No patients with juvenile type of diabetes were included in these studies.

During observations it was noted in one instance that there was an appar-

ent "hang-over" effect on blood sugars from the chlorpropamide given forty-eight hours previously. The possibility that intermittent therapy is feasible is suggested, and is being studied. Normal needle biopsy liver tissues, before therapy and at the time one patient developed a positive cephalin-cholesterol flocculation reaction, show a dissociation between biopsy results and liver function tests. With available tests, hepatotoxicity is extremely difficult to assess.

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COMPARATIVE HYPOGLYCEMIC EFFECTS OF CHLORPROPAMIDE, TOLBUTAMIDE, AND FURFURYLUREA*

T. S. Danowski and F. M. Mateer

*Section on Endocrinology and Metabolism, University of Pittsburgh School of Medicine, and
The Shadyside and Medical Center Hospitals, Pittsburgh, Pa.*

This study of the relative potency of the several hypoglycemic agents in altering the blood sugar and serum electrolytes in diabetes mellitus also permits some speculation on the possible sites of action in individual patients.

Materials and Methods

Observations were made on adult as well as juvenile diabetic patients. Age at the onset of diabetes and at the time of these studies, the sex of the patient, and the dosage and type of insulin taken, if any, are recorded in TABLE 1.

Chlorpropamide, tolbutamide, or furfurylurea,† 3.0 or 1.0 gm., was administered after an overnight fast to 9 juvenile and 17 adult diabetics. The venous blood sugar and the levels of inorganic phosphorus, CO₂, potassium, sodium, and chloride in serum from venous blood were measured at hourly intervals for 5 hours in control studies made both prior to and after the ingestion of the hypoglycemic agent. One week or more elapsed between the administration of chlorpropamide, tolbutamide, or furfurylurea. All studies were conducted on an outpatient basis after maintaining the subjects on rapid-acting insulin for 2 or more days.

Results

Control studies. From the control observations (FIGURE 1) it may be seen that in most patients the blood sugar levels remained relatively close to the zero value or declined slightly. In a few patients a distinct spontaneous rise or a definite fall, that is, 40 mg. per cent or more, was recorded (FIGURE 1). These limits were selected because such changes were rare in the control periods; there were but 2 such patients in each category. These were not confined to either the juvenile or adult group and were unrelated to either the type of insulin employed prior to these studies or to the usual daily dosage of insulin.

Chlorpropamide effects (3.0-gm. dosage). Chlorpropamide decreased the fasting blood sugar levels 40 mg. per cent or more in 14 of the patients, including 2 of the juvenile diabetics. In 2 others, 1 adult and 1 juvenile-type diabetic, the blood sugar rose by 40 mg. per cent or more. Decreases were

* The study reported in this paper is part of the Pittsburgh Plan for Heart Disease Control, and was aided by grants from the John A. Hartford Foundation, Inc., New York, N. Y.; the Public Health Service, Bethesda, Md.; and Chas. Pfizer & Co., Inc., Brooklyn, N. Y.

† The structural formula of furfurylurea is 1-*p*-tolylsulfonyl-3-furfurylurea. This compound was supplied by The National Drug Company, Philadelphia, Pa.

recorded most consistently in patients in whom the starting blood sugar was between 120 and 260 mg. per cent (FIGURE 2).

Comparisons of chlorpropamide, tolbutamide, and furfurylurea effects at 3.0 and 1.0-gm. dosages. Seven of the patients given 3.0 gm. of chlorprop-

TABLE 1

Patient	Sex	Age, years	Age at onset of diabetes, years	Usual insulin dosage, U.
Juveniles				
W. C.	M	13	10	25 (NPH)
N. G.	M	13	12½	3 (NPH)
R. L.	M	33	10	24 (Protamine Zinc I) 54 (Regular)
D. M.	M	14	13	No insulin
E. M.	F	16	12	46 (Lente)
S. M.	M	10	7½	30 (Crystalline)
L. P.	M	16	16	12 (NPH)
C. R.	F	21	2	65 (Protamine Zinc)
D. S.	M	16	13	40 (Protamine Zinc) 60 (Regular)
Adults				
G. C.	F	32	25	18 (NPH)
W. E.	M	32	20	50 (NPH)
J. E.	F	24	1	42 (Protamine Zinc) 22 (Regular)
A. H.	F	60	60	No insulin
P. K.	F	76	70	15 (Protamine Zinc)
A. L.	F	43	27	44 (NPH)
A. M.	F	63	63	No insulin
M. M.	F	49	40	25 (Lente)
B. P.	F	56	46	30 (NPH)
J. S.	F	56	56	No insulin
R. N.	F	54	46	No insulin
H. E.	F	55	47	No insulin
L. Y.	F	58	48	No insulin
C. S.	F	56	55	No insulin
J. A.	F	56	56	No insulin
L. R.	M	61	57	No insulin
A. B.	F	56	49	No insulin

amide were subsequently tested with similar amounts of tolbutamide and of furfurylurea, and then with 1.0 gm. of chlorpropamide and of furfurylurea. The blood sugar changes obtained are shown in FIGURE 3. TABLE 2 is based on blood sugar responses greater or less than minus 40 mg. per cent together with an indication of the blood sugar at zero time.

From FIGURE 3 and TABLE 2 it is evident that 3.0 gm. of chlorpropamide or of furfurylurea exerted comparable effects on the blood sugar of the 7 patients,

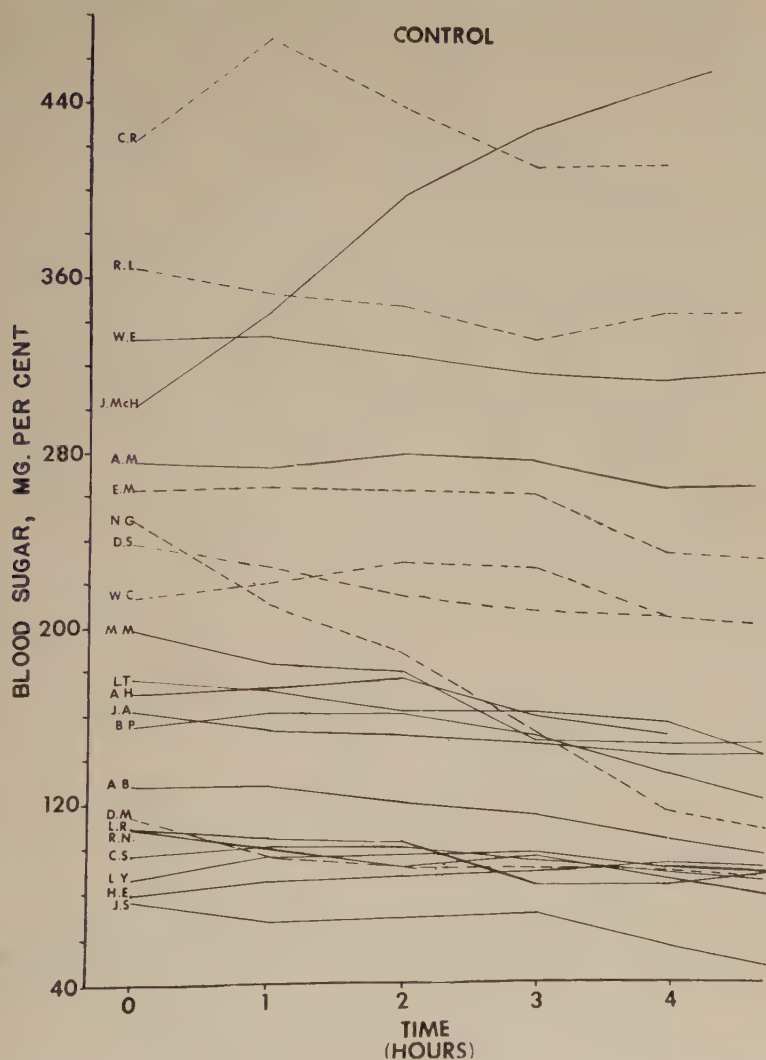


FIGURE 1. Serum blood sugar levels in series of adult and juvenile type diabetic patients. Dotted lines identify patients whose diabetes began during childhood. If a decrease in blood sugar of 40 mg. per cent or more is taken to be significant, then such a decrement occurred spontaneously in only 2 patients.

producing a decrease of 40 mg. per cent or more in 5. Tolbutamide in the same dosage produced similar effects in only 2 of the 7.

Again it should be noted that in general the responders, by the criterion employed—a fall of 40 mg. per cent or more—were patients with starting blood sugars of 120 to 260 mg. per cent, and that a greater proportion of values below 120 mg. per cent was present during the tolbutamide trials.

It should be noted further that of the agents and the dosages tested, only

furfurylurea lowered the blood sugar 40 mg. per cent or more in 3 of 5 patients with levels of 120 mg. per cent or less at zero time.

Finally, reducing the dosage of chlorpropamide from 3 gm. to 1 gm. produced a decrease of blood sugar of 40 mg. per cent or more in only 1 of the

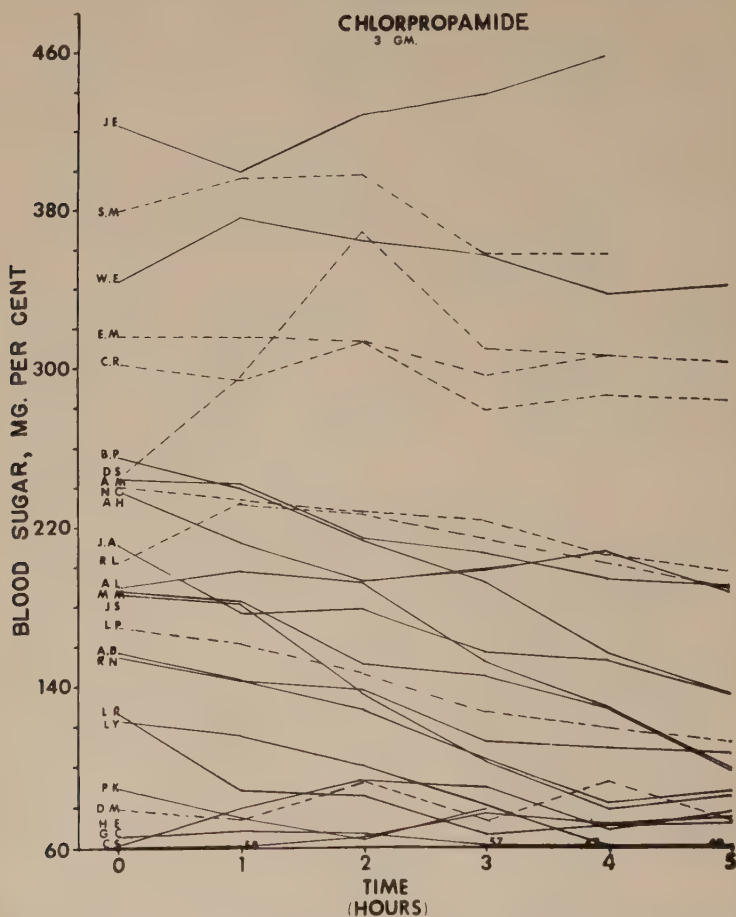


FIGURE 2. Hypoglycemic effects of chlorpropamide. The administration of 3.0 gm. of chlorpropamide at minus 45 min. produced decreases in blood sugar of 40 mg. per cent or more in 12 of the patients, including 2 juvenile diabetics.

patients. In 1 other, R. N., a change of this magnitude was partially cancelled by a subsequent rise. This contrasts with the 3.0-gm. studies in which all of the patients for whom such a comparison is available developed a distinct relative or absolute hypoglycemia. Furfurylurea, reduced to the dosage of 1.0 gm., decreased the responses to none of 6 in contrast to 5 of 7 with 3.0 gm.

Serum inorganic phosphorus changes in relation to blood sugar alterations. In FIGURE 4 the changes in blood sugar at 1, 2, 3, 4, and 5 hours in relation to

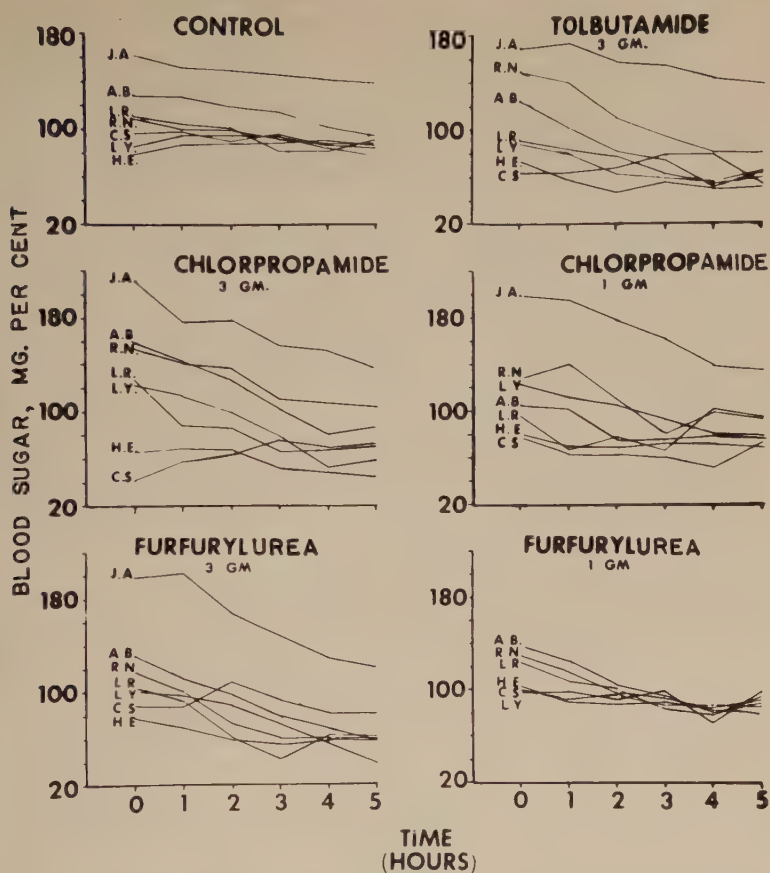


FIGURE 3. Comparison of hypoglycemic effects of tolbutamide, chlorpropamide, and furfurylurea in the same diabetic patients. Chlorpropamide at 3.0 gm. produced a decrease in blood sugar greater than 40 mg. per cent in 5 of 7 patients. A similar response in the same patients was obtained with 3.0 gm. of furfurylurea. Tolbutamide lowered the blood sugar in only 2 of the 7. It is noteworthy that the most consistent responses were obtained when the zero time blood sugar level was between 120 and 260 mg. per cent. Furfurylurea at 3.0 gm. may be the exception to this since it produced significant hypoglycemia with the starting blood sugar less than 120 mg. per cent.

the fasting levels have been plotted against the increment or decrement in serum inorganic phosphorus observed at these same times. If decreases of 0.4 mg. per cent or greater are considered, it is evident that in many patients such changes occurred without significant decreases—40 mg. or more—in the blood sugar levels. In a few, such decrements did accompany significant drops in blood sugar; in still other patients the blood sugar actually increased.

Discussion

Although the data presented indicate that not all patients are equally responsive to chlorpropamide or other oral insulin substitutes, decreases in

blood sugar can occur with chlorpropamide in both juvenile and adult-type diabetes. Insofar as relative efficacy in this regard is concerned, chlorpropamide and furfurylurea appeared to be more effective than tolbutamide in this small group of patients. It is noteworthy, too, that none of the other agents tested produced responses in patients unaffected by chlorpropamide (TABLE 2). Finally, although chlorpropamide and tolbutamide each appeared to be more effective when the zero value lay between 120 and 260 mg. per cent, furfurylurea alone acted at levels below 120 mg. per cent.

It is well established that serum inorganic phosphorus decreases quite regularly with the acceleration of carbohydrate metabolism in nondiabetic

TABLE 2
HYPOGLYCEMIC RESPONSES IN THE SAME ADULT DIABETIC PATIENTS TO
VARIOUS ORAL INSULIN SUBSTITUTES

Patient	Control	Chlorprop- amide 3.0 gm.	Chlorprop- amide 1.0 gm.	Tolbut- amide 3.0 gm.	Furfuryl- urea 3.0 gm.	Furfuryl- urea 1.0 gm.
J. A.	—	+	+	—	+	
A. B.	—	+	—*	+	+	—
R. N.	—*	+	—	+	+	—
L. R.	—*	+	—*	—*	+	—
L. Y.	—*	+	+	—*	+	—*
H. E.	—*	—*	—*	—*	—*	—*
C. S.	—*	—*	—*	—*	—*	—*
Blood sugar more than —40 mg. per cent	0/7	5/7	2/7	2/7	5/7	0/6

* Zero blood sugar less than 120 mg. per cent.

patients by glucose, insulin, or glucose and insulin in combination, and upon insulin injection in those diabetic patients previously shown to be unable to dispose of glucose loads at a normal rate and to develop a significant drop in inorganic phosphorus.¹ This decrease in serum inorganic phosphorus can be taken to reflect disposal of glucose by anaerobic glycolysis and formation of high-energy phosphorous compounds, since deposition of liver glycogen does not segregate phosphate.²⁻⁵

It may be assumed that diabetic patients maintained on diet alone possessed some ability to elaborate insulin. It would then appear, from those cases in which no significant decrease was observed in the serum inorganic phosphorus concomitant with the decrease in blood sugar, that chlorpropamide and the other agents tested had not increased anaerobic glycolysis (FIGURE 4). The observed decreases in blood sugar without concomitant hypophosphatemia are to be attributed, therefore, to decreased glycogenolysis and gluconeogenesis or increased deposition of glucose as liver glycogen, or both, a process that, unlike anaerobic glycolysis, does not pre-empt inorganic phosphorus from the serum. In patients in whom such hypoglycemic changes occurred with a concomitant drop in inorganic phosphorus, anaerobic glycolysis

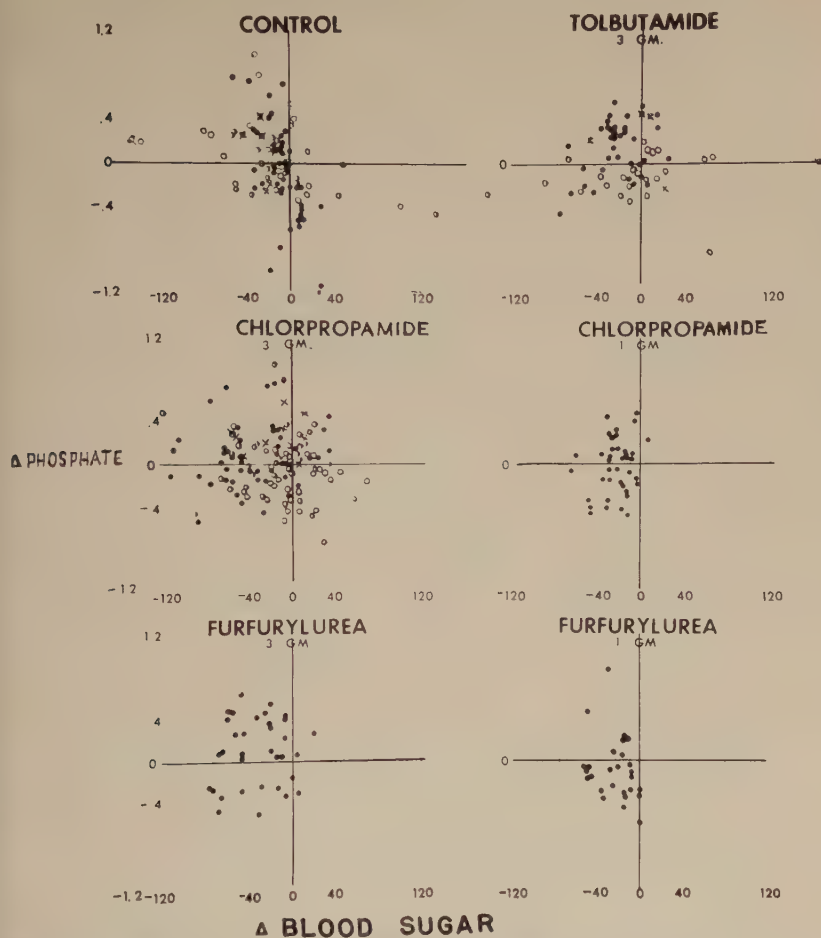


FIGURE 4. Correlation of serum inorganic phosphorus and blood sugar changes in patients receiving various oral insulin substitutes. The closed circles identify adult diabetics not on insulin. The half-closed circles represent adult diabetics on insulin, which was omitted on the day of the test. Open circles are for juvenile diabetics, and open circles with a superimposed cross identify juvenile diabetics not requiring insulin. It is apparent that on many occasions the blood sugar decreased without concomitant significant fall in serum inorganic phosphorus, that is, 0.4 mg. per cent or more. These decreases may be presumed to be instances of hypoglycemic effects resulting from decreased gluconeogenesis or segregation of glucose as hepatic glycogen. Neither of these processes preempts serum inorganic phosphorus. In patients in whom a significant decrease in blood sugar was accompanied by significant decrease in phosphorus, increased anaerobic glycolysis probably occurred. This could result from increased cell permeability, from a discharge of endogenous insulin, or from accentuation or prolongation of the effect of such endogenous insulin.

presumably was accelerated either by discharging insulin, by accentuating its action or survival, or by increasing the permeability of cells to glucose.

These comparisons are not offered, of course, with any assurance that the drugs tested would necessarily prove effective in reducing the insulin requirement or in regulating the diabetes in these patients. Also, it is possible that

in keeping with the experience of others with agents other than chlorpropamide, some of the patients who did not show a positive response to a particular drug initially might do so after several weeks of treatment.⁶

Summary and Conclusions

It is possible that responses to single dosages of oral hypoglycemic agents may differ from those obtained after repeated or prolonged therapy. The following statements therefore apply only to the single-dosage experiments herein described.

Certain diabetics did not respond to single dosage of chlorpropamide, tolbutamide, or furfurylurea with a decrease in blood sugar.

Chlorpropamide administered in 3-gm. doses at minus 45 min. produced, during the next 5 hours, a decrease of 40 mg. per cent or more in the zero blood sugar level of most of the adult diabetics and in 2 of 7 juvenile diabetics or patients whose diabetes began during childhood. During control observations, comparable decreases occurred only occasionally.

Chlorpropamide and furfurylurea tested in equal dosages in the same patients produced greater response than did tolbutamide.

Decreases with any of the agents tested were noted most consistently in patients in whom the zero blood sugar level lay between approximately 120 and 260 mg. per cent.

Furfurylurea may differ from the other agents in possessing a greater ability to lower blood sugar levels when the starting values are less than 120 mg. per cent.

The absence of significant decreases in the serum inorganic phosphorus in patients in whom the blood sugar fell significantly would suggest that in general the hypoglycemic responses in this group of diabetics are attributable to decreased gluconeogenesis and glycogenolysis or to segregation of glucose in the liver as glycogen, a process that, unlike anaerobic glycolysis, does not lower serum inorganic phosphorus. In those in whom both the blood sugar and the serum inorganic phosphorus fell, an increase in anaerobic glycolysis can be postulated—this as a result of a greater output, survival, or effectiveness of endogenous insulin, or as a consequence of an increase in the permeability of cells to glucose.

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CHLORPROPAMIDE IN SINGLE DOSAGE AND THE RESPONSES TO INSULIN OR GLUCOSE IN DIABETICS: THE ABSENCE OF EVIDENCE OF INCREASED GLUCOSE UTILIZATION*

T. S. Danowski, M. Leinberger, F. M. Mateer

Section on Endocrinology and Metabolism, University of Pittsburgh School of Medicine, and The Shadyside and Medical Center Hospitals, Pittsburgh, Pa.

In responsive patients with diabetes mellitus, chlorpropamide in single dosage lowers blood sugar levels, often without a concomitant decrease in serum inorganic phosphorus.¹ This suggests that, in patients who develop relative or absolute hypoglycemia after a single dose without significant

TABLE 1

Patient	Sex	Age, years	Ages at onset of diabetes, years	Usual insulin dosage, U.
Juveniles				
N. G.	M	13	12½	3 (NPH)
R. L.	M	33	10	24 (Protamine Zinc I)
				54 (Regular)
D. M.	M	14	13	No insulin
E. M.	F	16	12	46 (Lente)
S. M.	M	10	7½	30 (Crystalline)
L. P.	M	16	16	12 (NPH)
C. R.	F	21	2	65 (Protamine Zinc)
D. S.	M	16	13	40 (Protamine Zinc)
				60 (Regular)
Adults				
G. C.	F	32	25	18 (NPH)
W. E.	M	32	20	50 (NPH)
A. L.	F	43	27	44 (NPH)
M. M.	F	49	40	25 (Lente)
B. P.	F	56	46	30 (NPH)
J. McH.	M	37	21	28 (Regular)
J. S.	F	56	56	No insulin

hypophosphatemia, the chief or, perhaps, sole action of this agent is segregation of glucose as glycogen (a process not associated with deposition of phosphate) or suppression of gluconeogenesis or glycogenolysis. To examine further the possibility that there is a simultaneous increase in glucose utilization, glucose tolerance tests and insulin tolerance tests with and without

* The study reported in this paper is part of the Pittsburgh Plan for Heart Disease Control, and was aided by grants from the John A. Hartford Foundation, Inc., New York, N. Y., the Public Health Service, Bethesda, Md., and Chas. Pfizer & Co., Inc., Brooklyn, N. Y.

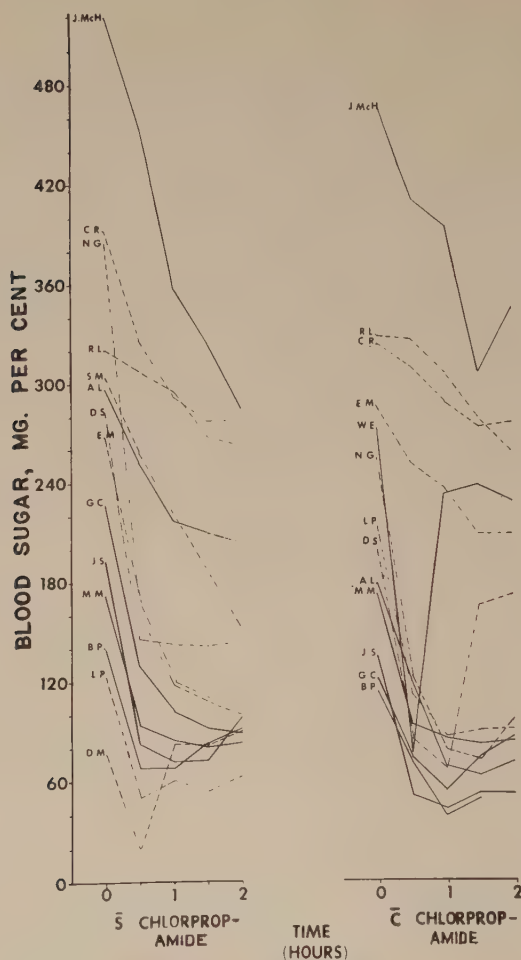


FIGURE 1. Blood sugar changes during insulin tolerance test in diabetics with and without 3.0 gm. of chlorpropamide at minus 45 min. Comparison of the changes at 0, $\frac{1}{2}$, 1, $1\frac{1}{2}$, and 2 hours in the 2 groups shows no accentuation of hypoglycemia after chlorpropamide (TABLE 2). Broken lines identify juvenile diabetics.

chlorpropamide administration in single dosage were undertaken, and the changes in serum inorganic phosphorus and other electrolytes were measured.

Materials and Methods

The clinical histories of the diabetic patients studied (some with juvenile diabetes mellitus and others with diabetes that began during the adult years) are summarized in TABLE 1. In most of the glucose tolerance tests, 0.5 gm. of glucose per kilogram of body weight was administered intravenously at zero time as a 50 per cent solution in water. In a few patients, 1.75 gm. of glucose per kilogram of body weight was used orally. In the

insulin tolerance tests, 0.1 U. of commercial rapid-acting crystalline insulin (per kilogram of body weight), diluted 1:9 with normal saline for ease of measurement, was injected intravenously at zero time. Chlorpropamide (3.0 gm.) was then given 45 min. before the start of the second glucose or insulin tolerance test. Blood sugar and serum electrolytes were measured just prior to and at intervals after the administration of the sugar or the insulin.

TABLE 2
STATISTICAL EVALUATION OF BLOOD SUGAR AND SERUM INORGANIC PHOSPHORUS
CHANGES DURING INSULIN TOLERANCE TESTS IN DIABETIC SUBJECTS

Period	Hours	No. of patients	Mean	±S.D.
Blood sugar				
Control.....	½	12	-84.9*	35.9
Chlorpropamide.....	½	12	-57.7	39.8
Control.....	1	12	-110.8	43.6
Chlorpropamide.....	1	12	-87.8	42.6
Control.....	1½	12	-119.8	46.0
Chlorpropamide.....	1½	12	-89.7	47.9
Control.....	2	11	-126.8	51.8
Chlorpropamide.....	2	11	-88.8	42.6
Serum inorganic phosphorus				
Control.....	½	10	-0.590	0.445
Chlorpropamide.....	½	10	-0.451	0.256
Control.....	1	10	-0.649	0.388
Chlorpropamide.....	1	10	-0.440	0.391
Control.....	1½	11	-0.509	0.423
Chlorpropamide.....	1½	11	-0.397	0.434
Control.....	2	7	-0.570	0.322
Chlorpropamide.....	2	7	-0.294	0.416

* Difference is statistically significant.

Results

Insulin tolerance tests. FIGURE 1 summarizes the blood sugar changes in the insulin tolerance tests with and without the prior administration of 3.0 gm. chlorpropamide at minus 45 min. Patients whose diabetes began during childhood are indicated by broken lines. The solid lines identify adult diabetics, all of whom, save J. S., usually required insulin in the dosages listed in TABLE 1. Inspection of the curves as a group, comparison of the blood sugar changes under both circumstances in individual patients (FIGURE 1), and statistical comparisons at the 30-, 60-, 90- and 120-min. points (TABLE 2) indicate that chlorpropamide did not accentuate the hypoglycemic effect of insulin during or at the termination of the glucose tolerance test. This was equally true of the juvenile or formerly juvenile diabetics and of the

adult diabetics. In fact, the blood sugar decrease at one-half hour was somewhat less after chlorpropamide (TABLE 2). It is probable that the week-to-week fluctuations in the starting blood sugar levels obscured the decrement often produced in fasting levels by chlorpropamide. Nevertheless, the fact

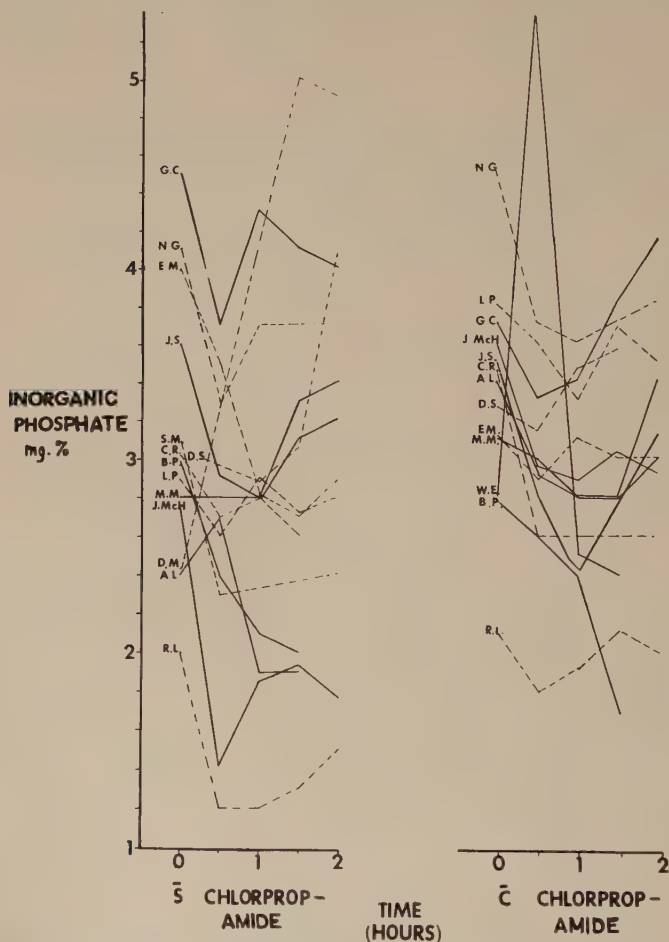


FIGURE 2. Serum inorganic phosphorus changes during insulin tolerance test in diabetics with and without 3.0 gm. of chlorpropamide. As in the blood sugar changes there is no evidence that the serum inorganic phosphorus responses are regularly affected by chlorpropamide (TABLE 2).

that insulin preceded by chlorpropamide failed to produce a decrement in blood sugar greater than that seen with insulin alone argues against an augmentation of insulin action by this oral insulin substitute when it is given in single dosage. It also eliminates the possibility that a single dosage of chlorpropamide enhanced endogenous insulin output in these particular

patients; it provides no information, of course, on the effects of repeated doses or prolonged therapy.

Chlorpropamide did not increase the magnitude of the hypophosphatemia produced by insulin alone (FIGURE 2, TABLE 2). Since increased utilization of glucose by means of anaerobic glycolysis increases the drop in serum inorganic phosphorus, the apparent absence of such a change in these experiments can be cited as further evidence against a release of endogenous insulin

TABLE 3
STATISTICAL EVALUATION OF BLOOD SUGAR AND SERUM INORGANIC PHOSPHORUS
CHANGES DURING I.V. GLUCOSE TOLERANCE TESTS IN DIABETIC SUBJECTS

Period	Hours	No. of patients	Mean	± S.D.
Blood Sugar				
Control.....	1½	8	124.5	28.3
Chlorpropamide.....	1½	8	88.1*	59.4
Control.....	1	8	104.0	51.4
Chlorpropamide.....	1	8	68.0	60.2
Control.....	1½	8	70.1	58.5
Chlorpropamide.....	1½	8	52.2	68.2
Control.....	2	8	52.1	58.9
Chlorpropamide.....	2	8	38.5	66.7
Serum inorganic phosphorus				
Control.....	1½	8	-0.228	0.206
Chlorpropamide.....	1½	8	-0.064*	0.190
Control.....	1	7	-0.250	0.253
Chlorpropamide.....	1	7	-0.011*	0.158
Control.....	1½	8	-0.262	0.263
Chlorpropamide.....	1½	8	-0.184	0.220
Control.....	2	8	-0.322	0.224
Chlorpropamide.....	2	8	-0.136*	0.281

* Differences are statistically significant.

or prolongation or potentiation of the action of exogenous insulin by chlorpropamide in single dosage.

Intravenous glucose tolerance tests. The administration of chlorpropamide 45 min. before the intravenous glucose produced a decrease in the increment in the blood sugar at the half-hour point (TABLE 3).

The administration of chlorpropamide decreased the usual hypophosphatemic response to glucose loads. The comparisons of the actual changes in blood sugar and serum inorganic phosphorus recorded during the glucose tolerance tests are summarized in TABLE 3. The differences in the blood sugar at the half-hour, and in the serum inorganic phosphorus at the half-, one-, and two-hour points are statistically significant.

It would appear that under the conditions of these experiments chlorpropamide did reduce the blood sugar response at the half-hour point, but it

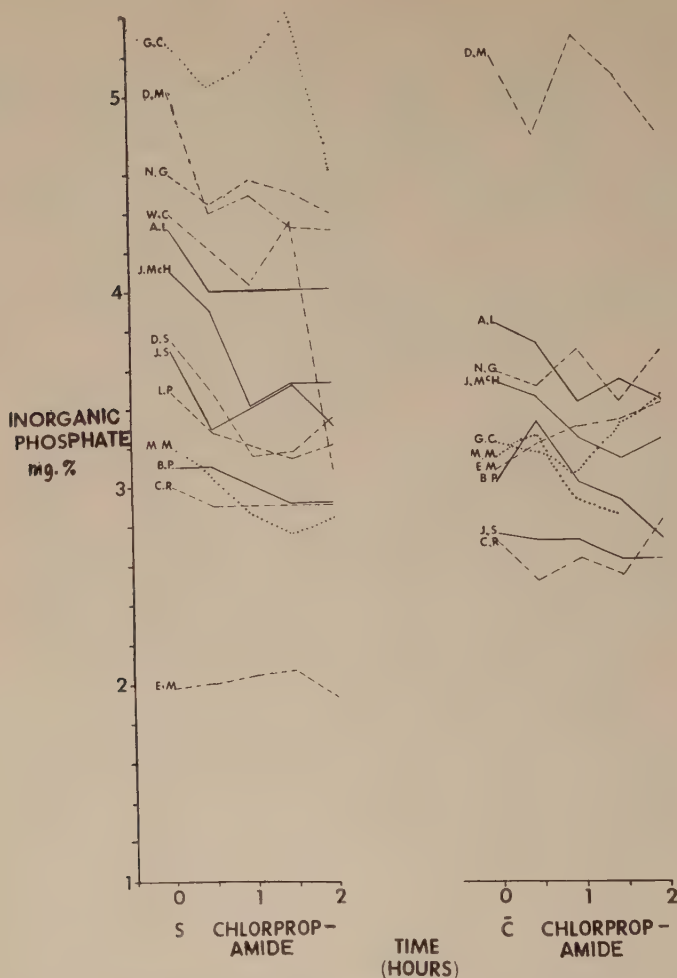


FIGURE 3. Blood sugar changes during glucose tolerance tests in diabetics with and without 3.0 gm. of chlorpropamide at minus 45 min. Solid lines identify adult diabetics and broken lines juvenile diabetics who received 0.5 gm. of glucose intravenously at zero time. Dotted lines represent patients who received 1.75 gm. of glucose per kilogram of body weight by mouth at zero time. In the intravenous glucose tolerance tests, chlorpropamide decreased the degree to which the blood sugar rose at the half-hour point (TABLE 3).

failed to increase the degree of hypophosphatemia. The latter can be advanced as an argument against discharge of any significant amount of endogenous insulin in these patients or potentiation of its action after chlorpropamide in single dosage.

The pattern of serum potassium, CO₂ content, sodium, and chloride during the insulin and the glucose tolerance tests, with and without chlorpropamide. Insulin tolerance tests in diabetics generally lower the serum potassium and raise

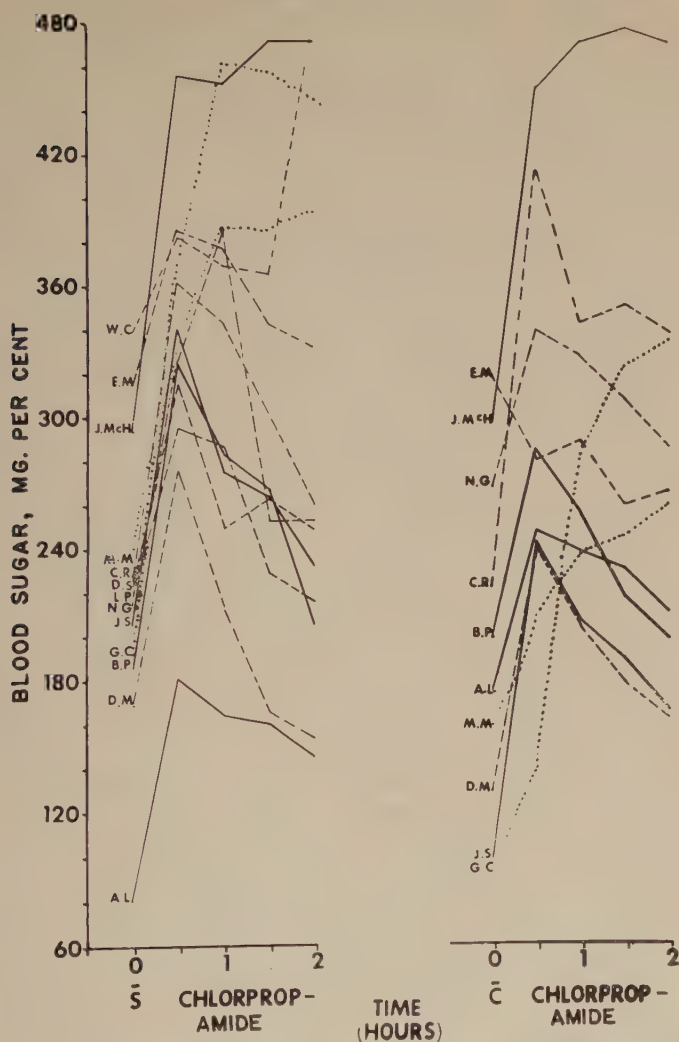


FIGURE 4. Serum inorganic phosphorus changes during glucose tolerance test with and without 3.0 gm. of chlorpropamide. Symbols are as identified in FIGURE 3. The serum inorganic phosphorus response to intravenous glucose was less after chlorpropamide (note the $\frac{1}{2}$, 1-, and 2-hour points in TABLE 3).

the serum total CO_2 content.² In diabetics not receiving insulin, the administration of glucose intravenously tends to raise the serum potassium and lower the serum total CO_2 content.² These responses were not altered by chlorpropamide administration.

Discussion

In these studies, in keeping with findings on analysis of tissues,⁵⁻⁶ it has been assumed that deposition of liver glycogen pre-empts potassium but not

inorganic phosphorus. The concomitants of muscle and other tissue glycogen formation are not known. If it is true that glycogen deposition does not segregate inorganic phosphorus, then the decrease in serum inorganic phosphorus reflects anaerobic glycolysis in cells and indicates either the formation of glucose-phosphates or high-energy phosphorus compounds, or some other metabolic process that pre-empt this ion.

In the studies herein described there was no evidence that the hypophosphatemic action of insulin or of glucose was enhanced by chlorpropamide in single dosage. This finding argues against either a direct action of chlorpropamide in single dosage on anaerobic glycolysis or an effect upon exogenous or endogenous insulin. The latter point is perhaps debatable, since no direct information is available on the endogenous supplies of this hormone in our patients. However, it should be pointed out that in those of our subjects whose diabetes was controlled without insulin or with insulin in tiny amounts, a significant residual capacity to produce insulin can be postulated. However, even these failed to show a greater drop and, therefore, no evidence can be brought forth in support of chlorpropamide action on the beta cells or on insulin secretion when the drug was administered in single dosage.

Published studies designed to clarify the mechanism or mechanisms of action of the earlier oral hypoglycemic agents have at times led to diametrically opposed conclusions. These and other discrepancies are undoubtedly attributable to variables such as species differences, dosage and schedule variations, and the characteristics of individual diabetics. Confining ourselves to measuring the responses to a single and often large dosage of an oral hypoglycemic agent of the sulfonylurea type, administered orally 45 min. before the test procedure at intervals of 1 week or more, we have found that the hypoglycemic responses observed under these circumstances are frequently unaccompanied by a decrease in serum inorganic phosphorus. This argues against a significant component of insulin discharge, insulin potentiation, insulin survival, or direct action on cells in those instances of hypoglycemia, since certainly at least the first three of these, present in sufficient degree, would be expected to lower the serum inorganic phosphorus. Although the indirect nature of the evidence offered must be admitted, the possibility is raised that the oral hypoglycemia agents of the sulfonylurea type elicit at least a 2-phase response. Initially, they act to suppress glycogenolysis and gluconeogenesis and segregate glucose as glycogen. Endogenous insulin released in amounts too small to affect serum inorganic phosphorus could play a role in this, if such an action of insulin is accepted. With continued therapy the beta cells are stimulated to elaborate insulin in quantities sufficient to produce the expected changes in serum inorganic phosphorus. Such a sequence of events would account for some of the discrepancies in published reports of the effects or lack of effects of sulfonylureas on serum inorganic phosphorus or on other evidences of endogenous insulin production.

Summary and Conclusions

In view of the fact that deposition of hepatic glycogen does not segregate phosphate in the liver, the decrease in serum inorganic phosphorus which, in

nondiabetics, invariably accompanies acceleration of carbohydrate metabolism by exogenous insulin or by glucose loads, can be taken as an index of enhanced disposal of glucose by anaerobic glycolysis.

The failure to observe any increment in the hypophosphatemia characteristic of insulin or of glucose tolerance tests in these particular diabetic patients following chlorpropamide suggests that chlorpropamide, in single dosage at least, did not accelerate anaerobic glycolysis either directly, by evoking endogenous insulin secretion, or by accentuating the action or survival of either exogenous or endogenous insulin. Of course, this does not exclude such a response in other diabetics nor the possibility that repeated or prolonged therapy might provide evidence of an effect of chlorpropamide on anaerobic glycolysis unrelated or related to insulin.

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CORTISONE ENHANCEMENT OF PERIPHERAL UTILIZATION OF GLUCOSE AND THE EFFECTS OF CHLORPROPAMIDE*

T. S. Danowski, F. M. Mateer, C. Moses

Section on Endocrinology and Metabolism, and the Addison H. Gibson Laboratory, University of Pittsburgh School of Medicine, and The Shadyside and Medical Center Hospitals, Pittsburgh, Pa.

Available evidence indicates that the hyperglycemia associated with excesses of ACTH and adrenocortical-type steroids, which is largely attributable to increased gluconeogenesis, may be based in part on a blockade of insulin action or glucose utilization in the tissue.¹⁻⁸ An extensive series of

TABLE 1
FASTING HYPOPHOSPHATEMIA PRODUCED BY THE ADMINISTRATION OF CORTISONE
45 MINUTES BEFORE TEST

Sex	Cortisone, mg.	Blood sugar, mg. %			Serum inorganic phosphorus*, mg. %		
		Mean	±S.D.	No. tested	Mean	±S.D.	No. tested
Healthy adults (21 to 60 years)							
M	0	84.3	11.1	64	3.43	0.54	67
M	200	81.5	11.3	60	3.23	0.56	61
F	0	80.7	11.6	71	3.58	0.55	70
F	200	81.9	12.4	64	3.37	0.50	65
Relatives of juvenile diabetics (21 to 60 years)							
M	0	91.5	13.2	62	3.32	0.59	60
M	200	87.9	10.9	26	2.97	0.49	26
F	0	85.0	11.6	69	3.41	0.56	67
F	200	84.5	9.6	27	3.12	0.46	27

* Differences between the bracketed groups are statistically significant.

glucose tolerance tests (with serum inorganic phosphorus measurements) conducted in this laboratory on relatives of juvenile diabetics, on apparently healthy persons, on healthy dental students, and on adult diabetics suggests that, under the conditions of our experiments, cortisone probably enhances the net utilization of glucose by anaerobic glycolysis as a result of increased gluconeogenesis, by stimulation or enhancement of insulin action, or by facilitating the entry of glucose (alone or in combination) into cells.

* The study reported in this paper is part of the Pittsburgh Plan for Heart Disease Control, and was aided by grants from the John A. Hartford Foundation, Inc., New York, N. Y., the Public Health Service, Bethesda, Md., and Chas. Pfizer & Co., Inc., Brooklyn, N. Y.

TABLE 2
BLOOD SUGAR AND SERUM INORGANIC PHOSPHORUS CHANGES DURING GLUCOSE TOLERANCE TESTS IN ADULTS, WITH AND WITHOUT CORTISONE ADMINISTRATION

CORTISONE ADMINISTRATION													
Sex		Cortisone	Blood sugar, mg. %				Serum inorganic phosphorus, mg. %						
			30 min.		2 hours		30 min.		2 hours				
			Mean	± S.D.	No. tested	Mean	± S.D.	No. tested	Mean*	± S.D.	No. tested		
Healthy adults (21 to 60 years)													
M	0	50.3	21.0	31	7.0	18.9	63	-0.233	0.353	30	-0.463	0.705	63
M	200	48.8	24.4	39	12.8	21.5	59	-0.302	0.166	34	-1.024	0.467	57
F	0	35.8	27.0	34	9.0	18.1	69	-0.279	0.326	33	-0.572	0.440	68
F	200	37.9	25.7	48	12.2	22.0	63	-0.378	0.269	45	-1.072	0.449	65
Relatives of juvenile diabetics (21 to 60 years)													
M	0	49.9	23.4	51	21.4	35.1	60	-0.324	0.328	48	-0.567	0.463	56
M	200	44.9	16.1	16	8.6	24.1	24	-0.334	0.338	16	-1.020	0.469	25
F	0	43.2	23.8	53	11.4	24.9	67	-0.303	0.385	53	-0.502	0.494	64
F	200	36.4	13.1	19	17.1	18.7	28	-0.087	0.430	19	-0.776	0.511	28

* Difference between the bracketed pairs of mean values are statistically significant.

Materials and Methods

Adult relatives of juvenile diabetics free of clinical symptoms of diabetes mellitus; apparently healthy adult male and female university personnel; healthy male dental students; and adult diabetics who did not require insulin received 1.75 gm. of glucose per kilogram of body weight with or without the administration, 45 min. earlier, of 200 mg. of cortisone by mouth. Blood

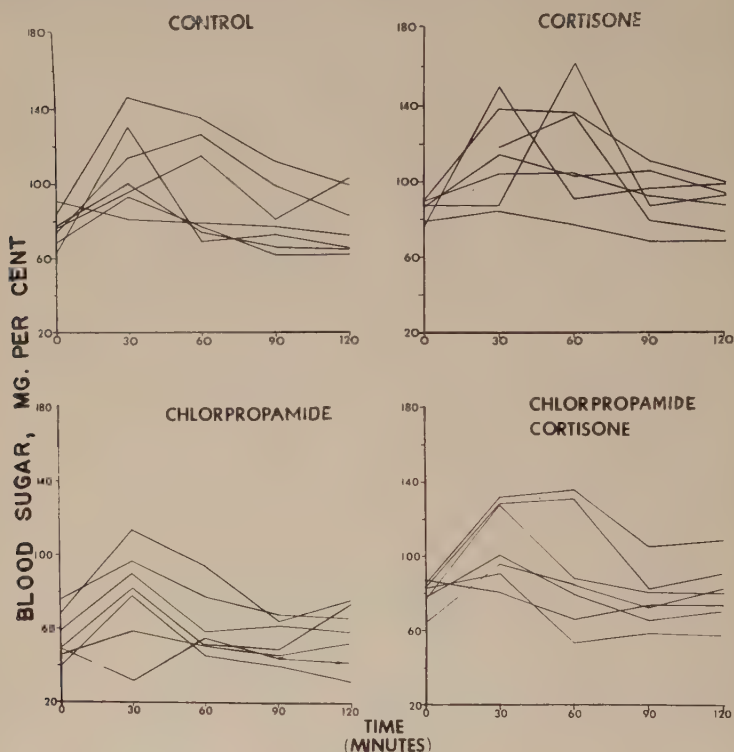


FIGURE 1. Blood sugar changes in healthy dental students during oral glucose tolerance test. Glucose was administered at 1.75 gm./kg. of body weight, with and without cortisone or chlorpropamide, alone or in combination.

for sugar and serum inorganic phosphorus analysis was withdrawn just prior to the administration of the glucose and at intervals thereafter, and was analyzed by methods in regular use in this laboratory.⁹ The studies with cortisone and chlorpropamide, alone or in combination, were limited to the dental students and to the adult diabetics.

Results and Comments

Fasting blood sugar and glucose tolerance tests with and without cortisone in healthy adults with and without diabetic offspring. TABLE 1 is made up of the mean fasting blood sugar and serum inorganic phosphorus values in healthy

university personnel and in relatives of juvenile diabetics in the 21- to 60-year age range with and without the prior administration of 200 mg. of cortisone 45 min. earlier. In addition to lower fasting blood sugar values in the healthy university personnel, both male and female (indicated by the single asterisk), it is noteworthy that the administration of cortisone invariably produced lower levels of serum inorganic phosphorus (measured 45 min.

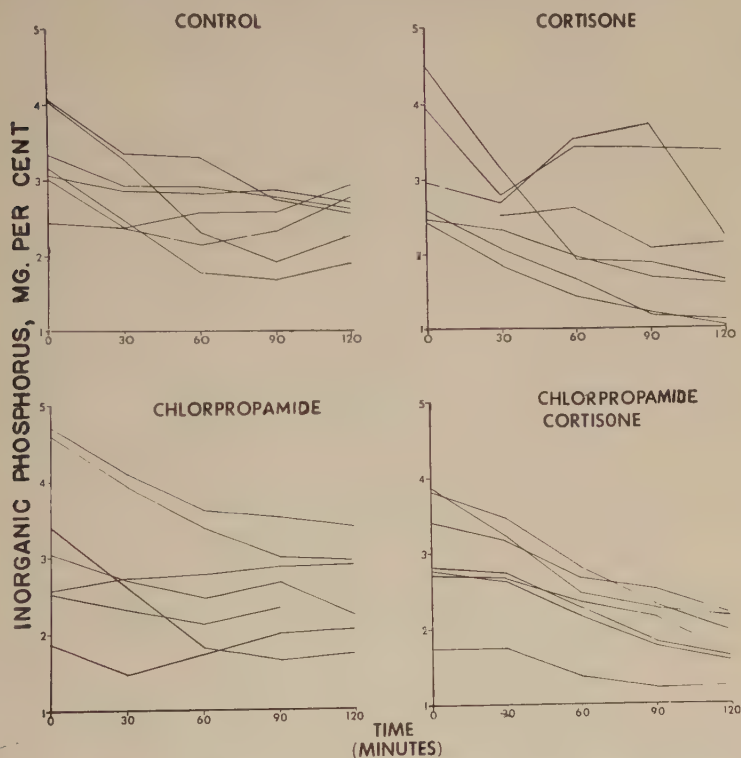


FIGURE 2. Serum inorganic phosphorus changes in healthy dental students during oral glucose tolerance test. Glucose was administered at 1.75 gm./kg. of body weight, with and without cortisone or chlorpropamide, alone or in combination.

later) without raising the blood sugar. The differences between the means are statistically significant and are indicated by a double asterisk.

The finding that cortisone lowered the serum inorganic phosphorus within 45 min. or less in these 2 groups suggests that this steroid enhances the extrahepatic utilization of glucose associated with a lowering of the serum inorganic phosphorus.³ This may result from (1) increased gluconeogenesis with comparable increased utilization of glucose by anaerobic glycolysis, which would prevent any rise in the blood sugar; (2) discharge of insulin or enhancement or prolongation of its action; (3) increased permeability of the cells to glucose; or (4) combinations of the above. In any circumstance, these data clearly indicate that the early effect of cortisone given to nondiabetic

adults is in all probability an enhancement rather than a blockade of glucose utilization.

The magnitude of the decline and the results of urinary analyses in selected patients indicated that the bulk of the observed decrease in serum inorganic phosphorus cannot be attributed to losses in urine.

TABLE 2 shows the levels of blood sugar and serum inorganic phosphorus found one-half hour and 2 hours following the administration *per os* of 1.75 gm. of glucose per kilogram of body weight at zero time with and without 200 mg. of cortisone given 45 min. earlier. In no instance did the steroid

TABLE 3
BLOOD SUGAR AND SERUM INORGANIC PHOSPHORUS CHANGES* DURING INTRAVENOUS
GLUCOSE TOLERANCE TEST FOLLOWING ADMINISTRATION OF CORTISONE,
CHLORPROPAMIDE, OR BOTH

	Cortisone	Chlorpropamide	Cortisone and chlorpropamide
Blood sugar.	No change	Lower initial and final sugars with usual rise. Blood sugar less than 60 mg. per cent reached in 4 of 7 patients. (Decreased gluconeogenesis or increased hepatic glycogenation without increased extrahepatic utilization.)	No change
Serum inorganic phosphorus decrement	Greater (Increased gluconeogenesis, enhanced insulin, or both.)	No change	Greater (In nondiabetic controls chlorpropamide did not alter cortisone effects.)

* Compared to controls.

affect the levels of blood sugar nor did it alter the serum inorganic phosphorus levels present at the 30-min. point. However, in each instance these amounts of cortisone greatly enhanced the usual hypophosphatemia, usually doubling the magnitude of the decrease.

As concluded from the observations on the fasting blood sugar, this steroid presumably increased the disposal of glucose by anaerobic glycolysis, by increasing gluconeogenesis, by discharging insulin or enhancing its effect, or by increasing cell permeability to glucose (alone or in combination).

Action of chlorpropamide upon cortisone hypophosphatemia during oral glucose tolerance tests in healthy dental students. From FIGURE 1 it is evident that the administration of cortisone to fasting healthy male dental students at minus 45 min. failed to modify the degree or the duration of the hyperglycemic response obtained with 1.75 gm. of glucose per kilogram of body weight given at zero time. On the other hand, the prior administration of

3 gm. of chlorpropamide to these same subjects at minus 45 min. (FIGURE 1) generally lowered the zero and final blood sugar concentrations as well as the zone through which the rises distributed themselves; this was done without modifying the magnitude of the actual increments. It is noteworthy, moreover, that hypoglycemia, that is, values below 60 mg. per cent, developed in 4 of 7 subjects. Finally, the combination of cortisone and chlorpropamide in the dosages and at the time cited (FIGURE 1) canceled the general lowering of the blood sugar curves noted with chlorpropamide alone, restoring them

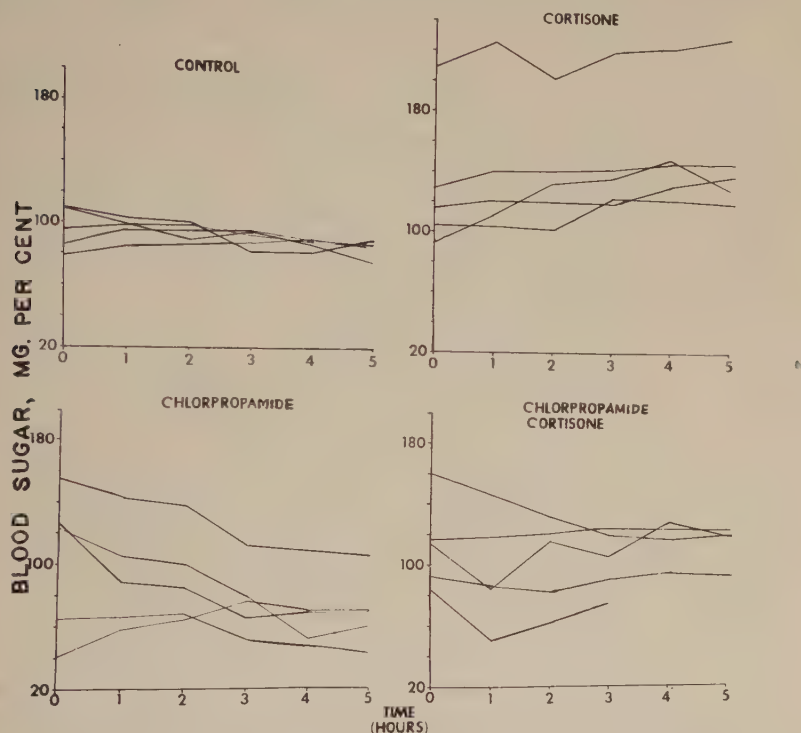


FIGURE 3. Blood sugar changes during oral glucose tolerance test. Glucose administered at 1.75 gm./kg. of body weight, in diabetics with and without cortisone or chlorpropamide, alone or in combination.

to the levels seen in the control studies or in those made with cortisone alone; certainly, the absolute hypoglycemia seen with chlorpropamide alone did not develop.

From FIGURE 2 it is evident that cortisone administration at minus 45 min. increased the hypophosphatemia that is a regular feature of such intravenous glucose tolerance tests in nondiabetic subjects. In the studies with chlorpropamide alone (FIGURE 2), in the majority of which the blood sugar reached absolute hypoglycemic levels, the serum inorganic phosphorus did not decrease beyond the range seen in the control observations and certainly did not fall as low as in the cortisone experiments. In the combination of

cortisone and chlorpropamide (FIGURE 2) the serum inorganic phosphorus changes resembled those seen with cortisone. The changes in the blood sugar and the serum inorganic phosphorus under these four circumstances are summarized in TABLE 3.

The greater degree of hypophosphatemia in the cortisone studies suggests that this steroid increased the utilization of glucose by anaerobic glycolysis, by discharging insulin, prolonging, or potentiating insulin action, or by

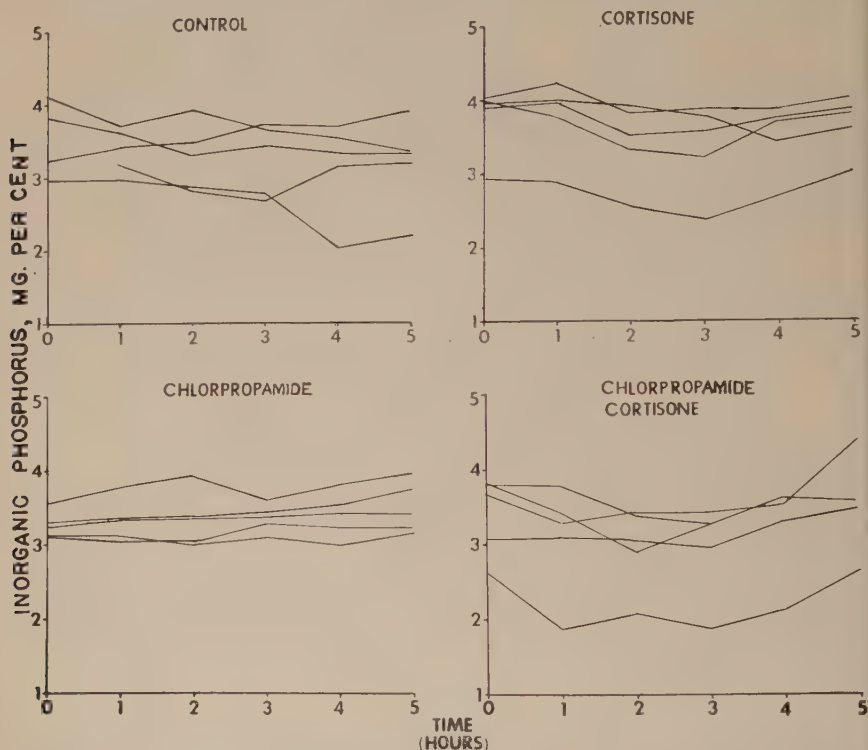


FIGURE 4. Serum inorganic phosphorus changes during oral glucose tolerance test. Glucose was administered at 1.75 gm./kg. of body weight, in diabetics with and without cortisone or chlorpropamide, alone or in combination.

increasing cell permeability. Increased gluconeogenesis could have occurred and potentiated this hypophosphatemic response by providing an increased glucose load.

The lower blood sugar levels at zero time, throughout the course of the glucose tolerance tests and at their end, suggest that chlorpropamide decreased gluconeogenesis or enhanced the supplies or action of insulin or both. However, the fact that the serum inorganic phosphorus changes were no greater than those seen in the control studies argues for the former as the predominant or sole action.

Cortisone and chlorpropamide effects in diabetics. FIGURES 3 and 4 show the blood sugar and serum inorganic phosphorus levels in diabetics during several samplings for five hours with or without the prior administration of cortisone or chlorpropamide, alone or in combination.

In contrast to the shorter studies in the healthy university personnel, in the relatives of diabetics, and in the healthy dental students the blood sugar levels tended to rise when cortisone was given alone (FIGURE 3). However, the same trend to hypophosphatemia noted in the three other groups was observed (FIGURE 4). Chlorpropamide alone tended to lower the blood sugar without decrease in the inorganic phosphorus (FIGURES 3 and 4).

In the chlorpropamide-plus-cortisone studies it appears that chlorpropamide tended to produce an initial lowering of the blood sugar in three of the subjects (FIGURE 3); this was maintained in only one. The serum inorganic phosphorus changes (FIGURE 4) assumed the pattern seen with cortisone alone.

Under the conditions of these experiments, chlorpropamide partially modified the hyperglycemia of cortisone administration, probably by suppressing gluconeogenesis. Since the serum inorganic phosphorus changes of the cortisone experiments were not accentuated, it may be argued that chlorpropamide did not exert any significant action on any endogenous insulin that these patients may have had. Alternatively, it is possible that a slight enhancement was evoked just sufficient to offset the decrease resulting from the lesser degree of gluconeogenesis.

Summary and Conclusions

Fasting blood sugar and glucose tolerance tests with and without cortisone in healthy adults and in relatives of juvenile diabetics. The administration of 200 mg. of cortisone 45 min. before an oral glucose tolerance test (1.75 gm./kg. of body weight) lowered the fasting and the 2-hour levels of serum inorganic phosphorus without discernible change in the control blood sugar concentrations or increments. This suggests that under the conditions of these experiments, in nondiabetic subjects, this steroid enhanced the utilization of glucose by anaerobic glycolysis, as reflected by significant increases in the usual hypophosphatemia. This could represent increased gluconeogenesis, increased secretion, intensification or prolongation of insulin action, or increased cell permeability to glucose, alone or in combination. These findings argue strongly against a peripheral blockade of insulin action in these experiments.

Action of chlorpropamide upon cortisone hypophosphatemia. Chlorpropamide administered to healthy nondiabetic male dental students 45 min. before an intravenous glucose tolerance test did not modify either the usual increment in blood sugar or the usual decrement in serum inorganic phosphorus, although the absolute levels of blood sugar at the start, during, and at the end of the tolerance test were significantly lowered, and hypoglycemia appeared in the majority.

The failure to observe a further decrease in the serum inorganic phosphorus during these glucose tolerance tests suggests that the chief mode of action of the chlorpropamide was decreased gluconeogenesis or increased removal of extracellular glucose to form hepatic glycogen. This is concluded since

both of these processes are known to lower the blood sugar without segregation of serum inorganic phosphorus.

Our observations suggest that, in the quantities and under the conditions employed, cortisone cancels the hypoglycemic effect of chlorpropamide, presumably by overriding the inhibition of gluconeogenesis by the latter, but that chlorpropamide does not interfere with the cortisone-enhanced hypophosphatemia.

Blood sugar and serum inorganic phosphorus changes indicate that cortisone, when first given to healthy subjects, enhances rather than decreases the net disposal of glucose by processes that preempt serum inorganic phosphorus.

Cortisone and chlorpropamide effects in diabetics. In adult diabetics not requiring insulin, 200 mg. of cortisone given *per os* at minus 45 min. tended to raise the venous blood sugar levels during several samplings for 5 hours, while chlorpropamide had the opposite effect. When the two were administered together, the blood sugar levels dropped initially in 3 of 5 subjects, but the decrease was maintained in only 1 of these. These findings suggest that, in the amounts given, chlorpropamide could not completely overcome the hyperglycemic action of cortisone.

Insofar as the serum inorganic phosphorus levels are concerned, no changes were observed with chlorpropamide, while with cortisone or with cortisone and chlorpropamide a tendency to decrease was noted. This again indicates that under these experimental conditions chlorpropamide did not cancel this action of cortisone.

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INFLUENCE OF CHLORPROPAMIDE UPON POSTPANCREATECTOMY AND SPONTANEOUS DIABETES*

Kermit L. Pines, Edgar Leifer, DeWitt Goodman

Department of Medicine, Columbia University College of Physicians and Surgeons, and the Presbyterian Hospital, New York, N. Y.

Despite the current uncertainty regarding the mechanism of the hypoglycemic effect of the aryl sulfonylureas, the observation of their failure to lower the blood sugar in severe alloxan diabetes in animals¹ and in depancreatized animals,² and man^{3, 4} remains unchallenged. Although negative results were anticipated because of the structural similarity of chlorpropamide to carbutamide, it was considered important to observe the action of the newer agent in pancreatectomized patients.

Two patients in whom complete pancreatectomy had been performed by Porter and his associates⁵ were selected. One was a 60-year-old woman, 6 years after the operation; the other, a 55-year-old man, 16 months after removal of the pancreas. In both, surgery had been performed for pancreatic carcinoma. Neither had evidence of recurrent or persistent tumor at the time of these studies.

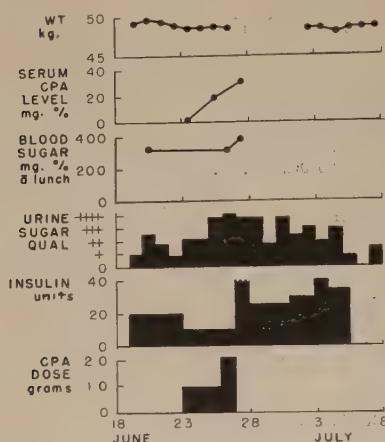
The patients were admitted to the hospital and given their regular diets plus NPH insulin for periods of 7 and 5 days, respectively. During these periods base-line measurements were obtained, including blood counts, urine analyses, tests of liver function (cephalin flocculation, thymol turbidity, serum bilirubin, serum alkaline phosphatase, serum cholesterol, and serum protein electrophoresis), and measurements of phenolsulfonphthalein excretion and blood urea nitrogen. Blood sugar levels were determined by the method of Folin and Wu at frequent intervals. Those obtained 3 hours after breakfast are shown in FIGURES 1 to 3. Urinary glucose was estimated 5 times daily by the qualitative Benedict's method; the mean daily value is shown in the figures. Serum chlorpropamide (CPA) concentrations were determined by the ultraviolet spectrophotometric method developed by Toolan and Wagner.⁶

Following the initial period, chlorpropamide was administered in a dose of 1 gm. daily and the insulin dose was simultaneously reduced from 20 to 10 U. of NPH in the first patient, M. B. (FIGURE 1). During the next 3 days, glycosuria increased markedly and the patient developed thirst and polyuria. The dose of chlorpropamide was raised to 2 gm. on the fourth day, after which it was discontinued owing to the appearance of nausea and vomiting. The serum chlorpropamide level at this time was 30 mg./100 ml. The first day following the experimental period, 80 U. of regular insulin were given. Satisfactory regulation was not accomplished, however, for more than 1 week.

The second patient, R. R. (FIGURE 2), was also given 1 gm. of chlorpropamide daily after the control period. The previous dosage of 40 U. of NPH

* The work reported in this paper was supported in part by a grant from Chas. Pfizer & Co., Inc., Brooklyn, N. Y.

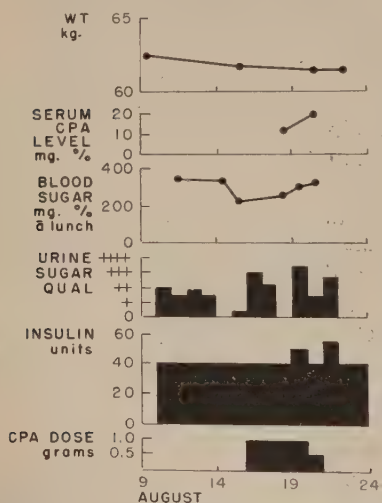
M.B. 60 ♀ #812519 PANCREATECTOMY 1952



	CONTROL	R _x	DAYS of R _x
HGB	12.8	11.3	4
WBC	8,100	8,100	4
N	54	59	4
L	44	39	4
M	2	2	4
URINE			
S.G.	1.010		
ALBUMIN	1+		
BUN	23	28	4
CEPH FLOCC	0	0	4
THYMOL TURBID	0	0	4
BILIRUBIN	TRACE	TRACE	4
ALK P'TASE	12 I KAU	16 KAU	4
CHOLESTEROL TOTAL	209	167	4
ESTERS	151	106	
PROTH TIME	14 sec	13	4
RAI TRACER	22%	16%	4

FIGURE 1. Response to chlorpropamide of patient M. B. six years after pancreatectomy.

R.R. 55 ♂ #1314651 PANCREATECTOMY 1957



	CONTROL	R _x	DAYS of R _x
HGB	12.0		
WBC	13,150	14,840	5
P	40	30	5
L	57	61	5
E	2	3	5
B	1	6	5
PLTS	385,000	459,000	4
URINE			
S.G.	1,004	1,020	5
ALBUMIN	0	±	5
GLUCOSE	0	4+	5
BUN	22		
PSP	75%		
ALK P'TASE	12.7 KAU	15.8 KAU	5

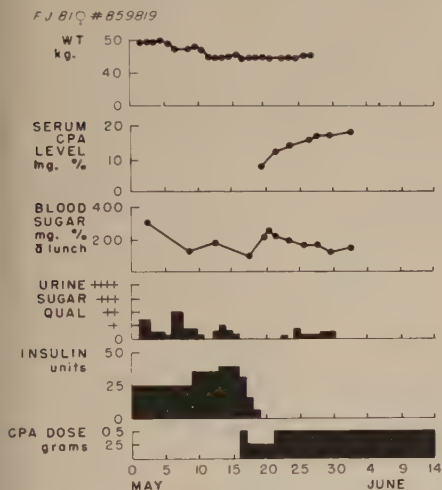
FIGURE 2. Response to chlorpropamide of patient R. R. sixteen months after pancreatectomy.

insulin was continued because of persistently elevated blood sugar levels. Despite the addition of chlorpropamide, with serum concentrations to 20 mg./100 ml., the blood sugar rose and glycosuria increased during the experimental period.

Laboratory studies (FIGURES 1 and 2) indicated the presence of a mild neutropenia of questionable significance, which increased in R. R. after chlorpropamide administration. Minimal albuminuria also was observed in this patient after treatment with this agent. The fall in serum cholesterol

in M. B. is difficult to evaluate because of major alterations in food intake during part of the period. The serum alkaline phosphatase concentrations increased in the 2 patients from 12.1 to 16, and from 12.7 to 15.8 King-Armstrong units (normal, 12 or less), respectively.

Chlorpropamide was also given to 6 patients with spontaneous, stable diabetes that began after 30 years of age. Among them were 5 women and 1 man ranging in age from 58 to 81 years and, in insulin requirement, from 20 to 60 U. Insulin was discontinued, and 0.5 to 1 gm. daily doses of chlorpropamide were given for periods of 2 weeks to 4 months. In 4 members of



	CONTROL	R _x	DAYS of R _x
HGB	13.5	11.8	28
WBC	11,000	8,200	28
N	89	72	28
L	6	26	28
M	4	2	28
E	0		
S	1		
BSP		10%	15
ALK P'TASE		10.1KAU	15
CEPH. FLOCC.	0	0	15
THYMOL TURB.	0	0	15
RAI TRACER	15%	19%	19

FIGURE 3. Response to chlorpropamide of a patient with spontaneous diabetes.

this group, blood sugar concentrations were maintained at 200 mg./100 ml. or less 3 hours after breakfast. FIGURE 3 summarizes the observations in a patient whose blood sugar levels were satisfactorily controlled by chlorpropamide alone. This agent replaced 30 U. of globin insulin in an obese 77-year-old woman, following an unsuccessful trial of tolbutamide.

Of the 2 patients who failed to respond, one was a 75-year-old woman with mild nephropathy after 22 years of diabetes. Neither tolbutamide in doses as high as 2 gm. daily, nor chlorpropamide at 0.5 gm. daily for 2 weeks could be substituted successfully for 40 U. of protamine zinc insulin. The other, a 68-year-old man well regulated on 20 U. of protamine zinc insulin, had also failed to respond to tolbutamide. Although an initial course of chlorpropamide restored the blood sugar values to essentially normal levels, a second trial of this agent after 1 week on tolbutamide was ineffective in preventing hyperglycemia.

It is apparent from the observations reported that chlorpropamide had no measurable hypoglycemic effect under the conditions of the study in 2 patients previously subjected to complete pancreatectomy. It should be emphasized that in neither was control of the blood sugar adequate prior to chlorprop-

amide administration. Parallel with previous observations on other aryl sulfonylureas, Root⁷ has reported the potentiation of insulin action by chlorpropamide in 2 totally depancreatized dogs. Such an effect was not found in the present study on 2 human subjects, although it should be noted that the agent was given for much shorter periods. In one of Root's dogs, the serum alkaline phosphatase increased and a fatty liver was found at autopsy. The significance of the small increases in alkaline phosphatase levels in the 2 patients reported here requires further evaluation. In the 6 patients with spontaneous diabetes no disturbance of liver or kidney function was seen during chlorpropamide treatment, nor was there evidence of alteration of the bone marrow or of allergic reaction.

Effect of Chlorpropamide upon Plasma Unesterified Fatty Acid Concentrations

The importance of plasma unesterified fatty acids (UFA or NEFA) in lipid transport and their relationship to carbohydrate metabolism have been

TABLE 1
PLASMA UNESTERIFIED FATTY ACID LEVELS FOLLOWING ORAL CHLORPROPAMIDE*

Time (min.)	Serum chlorpropamide				Blood sugar				Unesterified fatty acids			
	Patient				Patient				Patient			
	a†	b	c	d	a	b	c	d	a	b	c	d
	(mg./100 ml.)				(mg./100 ml.)				(mEq./l.)			
0	0	0	0	0	142	130	84	217	0.50	0.36	0.33	0.67
60				2.9	132	114	82	196	0.34	0.24	0.36	0.47
165	24				112	84	61	164	0.27	0.16	0.33	0.33
225		25	20		108	93	63	115	0.34	0.35	0.36	0.24
300		24	25	18		92	59	70		0.48	0.48	0.50

* Patients *a*, *b*, and *c* were given 3 gm. of chlorpropamide; patient *d* received 2 gm.

† Patient *a* took NPH insulin, 10 U. daily, until 48 hours prior to this experiment; patients *b*, *c*, and *d* required no insulin.

demonstrated by Dole,⁸ Gordon,⁹ and others. Elevated levels in diabetic patients in the postabsorptive state and during ketosis have been demonstrated by Bierman.¹⁰ The high UFA concentrations found in acidosis fall sharply in response to insulin. The hypoglycemic effect of tolbutamide is accompanied by a decline in plasma UFA concentrations.¹¹

In the present study, 4 patients with mild diabetes were given a single 2- or 3-gm. oral dose of chlorpropamide after a 9- or 14-hour period of fasting. Only 1 member of the group required insulin; this was discontinued 48 hours prior to the study. Plasma levels of sugar, chlorpropamide, and UFA were

determined at intervals for 5 hours. The method of Gordon¹² was used for UFA estimations.

The results are listed in TABLE 1. The blood sugar decreased in all patients as the serum chlorpropamide levels rose to 18 mg./100 ml. or more. The UFA concentration fell in 3 patients, was unchanged in 1 and, at the end of the study, rose in all.

Conclusions

Chlorpropamide, like carbutamide and tolbutamide, is an effective oral hypoglycemic agent in some patients with diabetes. Failure of chlorpropamide to produce a decrease in the blood sugar level in the absence of the pancreas was observed in two patients. A small increase in serum alkaline phosphatase levels was found in both pancreatectomized patients after treatment with chlorpropamide for brief periods. Plasma unesterified fatty acid levels decreased after chlorpropamide as they do after insulin administration.

Acknowledgment

We are indebted for technical assistance to Barbara Jones and Emily Bidwell, and also to Margaret Hawthorne of the Metabolism Ward of the Presbyterian Hospital.

Addendum

After preparation of this paper, 2 patients with spontaneous diabetes developed significant elevations of serum alkaline phosphatase concentrations while receiving 0.5-gm. doses of chlorpropamide daily. In one, withdrawal of chlorpropamide was followed by prompt return of the alkaline phosphatase to normal levels. The other patient, after continuing chlorpropamide in 0.25-gm. doses, developed a severe skin eruption and a cholangiolitic hepatitis, with recovery in 1 month.

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Discussion of the Paper

HENRY RICKETTS (*Department of Medicine, University of Chicago School of Medicine, Chicago, Ill.*): I think it is still quite mysterious that the human being without a pancreas and maintained on insulin fails to show a depression of blood sugar when sulfonylureas are added to the regimen, in contrast to the totally depancreatized dog in which (as shown by a number of workers, including ourselves) the addition of sulfonylureas to suboptimal doses of insulin results in a clear-cut depression of blood and urinary glucose. I have yet to hear why the human being responds differently from the dog in this respect, and I wish we could have the answer.

When speaking of the effects of sulfonylureas in depancreatized people or animals, we should be careful about our use of the term "potentiation of insulin." Insulin potentiation implies accentuation of the effects of insulin; this is not quite true in depancreatized dogs because, as we have demonstrated in a work thus far unpublished, in the totally depancreatized dog maintained on suboptimal amounts of insulin and receiving chronically added sulfonylureas, there is a clear reduction in blood and urinary sugar with no change of nitrogen excretion. Since the addition of 2 U. of insulin to the regimen in these animals produces the expected decline in blood and urinary sugar and, now, a clear diminution of nitrogen excretion, I do not think we can refer to the sulfonylurea effect as insulin potentiation. I suspect that it is not and I suspect also, if our evidence is valid, that one must conclude that in the dog without a pancreas the sulfonylurea effect occurs in other tissues for whose integrity a small minimum of insulin is necessary.

THE EFFECTS OF CHLORPROPAMIDE ON ENDOCRINE FUNCTION IN PATIENTS WITH DIABETES MELLITUS AND ITS EFFECTS IN OTHER ENDOCRINE DISORDERS*

George J. Hamwi, Thomas G. Skillman, Fred A. Kruger, Lucy R. Freedy
Department of Medicine, Ohio State University, Columbus, Ohio

The continued widespread use of tolbutamide for the treatment of diabetes mellitus has left little doubt about either the efficacy of action or the acceptance by both the physician and patient of this oral agent for the treatment of this disease. Similar drugs are destined to undergo investigation, and certain of these will find a place as competitors with, or substitutes for, tolbutamide.

In order to compete with tolbutamide a compound must be at least as potent a hypoglycemic agent and equally innocuous from the standpoint of toxicity. Additional attributes of such a drug might include equal effectiveness at lower dose ranges or blood levels, less expense to the patient, or usefulness as alternate therapy in patients known to be hypersensitive or unresponsive to tolbutamide.

It has been the aim of our study to accumulate data on chlorpropamide regarding each of these points. Preliminary results indicate that chlorpropamide is effective as an oral hypoglycemic agent in the same type of diabetic patient that responds to tolbutamide, and that dosages of approximately one third the magnitude of the latter drug result in equal or higher serum levels. Additional data suggest that hypoglycemic reactions are more common with chlorpropamide, but in short-term usage few manifestations of toxicity to vital organs were observed. As with other sulfonylurea derivatives, slight inhibition of thyroid function may occur, but this does not appear to be clinically significant.

Methods and Results

Fifty-four patients were selected for a trial of chlorpropamide therapy because, as a group, they characterized the diabetic most likely to respond to a sulfonylurea hypoglycemic agent as regards age, sex, body type, duration of diabetes, and daily insulin need. The group included 13 recently discovered diabetics who could not be controlled with diet, 13 individuals previously controlled with insulin, and 28 patients who had been treated with tolbutamide (TABLES 1, 2, and 3).

Chlorpropamide was administered orally in doses ranging from 0.2 to 1.0 gm. daily, and frequently a priming dose of 1 or 2 gm. was given. Blood sugar levels were obtained daily in both the fasting and postprandial states

* Work reported in this article was supported in part by Research Grant 2A-5118 (C) and (CS) from The National Institute of Arthritis and Metabolic Diseases, Public Health Service, Bethesda, Md.; by the Comly Coleman Fund of the Ohio State University, Columbus, Ohio; and by Chas. Pfizer & Co., Brooklyn, N. Y.

TABLE 1
METHOD OF STUDY OF HYPOGLYCEMIC EFFECT OF CHLORPROPAMIDE

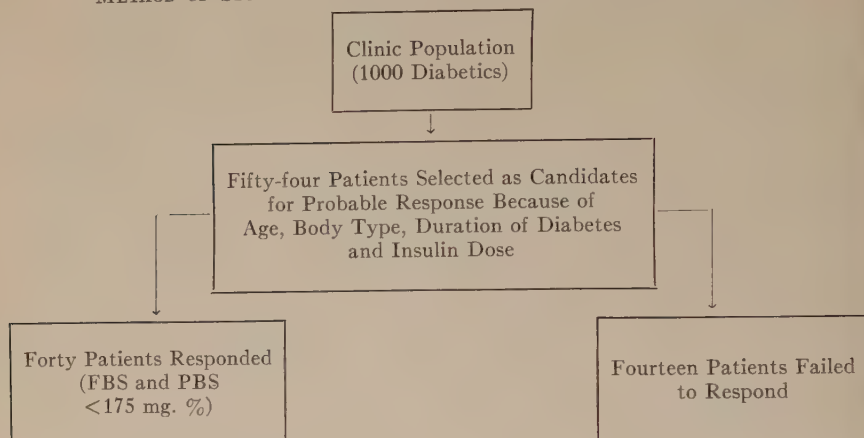


TABLE 2
CHARACTERISTICS OF RESPONSIVE AND NONRESPONSIVE PATIENTS

Factors	Responsive (40 patients)	Nonresponsive (14 patients)
Mean age (years).....	60	56
Percentage female.....	80	71
Percentage more than 20 lb. overweight.....	75	64
Duration of diabetes (years).....	5.6	6.4
Percentage with history of ketosis.....	3	29
Mean daily insulin (units).....	11	25

TABLE 3
TREATMENT BEFORE CHLORPROPAMIDE

	Responsive (40 patients)	Nonresponsive (14 patients)
Controlled on tolbutamide.....	13	0
Uncontrolled on tolbutamide.....	10	5
Uncontrolled on diet alone.....	10	3
Controlled with insulin.....	7	6

in hospitalized patients, and weekly or biweekly in outpatients. Patients were counted as responsive if all blood sugar levels fell below 175 mg. per cent and remained below this level. Chlorpropamide treatment was continued for at least 7 days in nonresponsive patients and, usually, until the serum level of the drug approached or exceeded 20 mg. per cent. Treatment was continued in all patients who responded, except in instances where untoward reactions developed.

Forty of the 54 patients met the criteria for response and 14 failed to do so. As a group, those responding were slightly older, more obese, and had a

TABLE 4
RESULTS OF CHLORPROPAMIDE TREATMENT IN PATIENTS FORMERLY TREATED
WITH TOLBUTAMIDE

Patients	Tolbutamide period		Chlorpropamide period		P 607 level (mg. %)
	Mean FBS (mg. %)	Daily dose (gm.)	Mean FBS (mg. %)	Daily dose (gm.)	
<i>Group 1</i> Response to both agents: 13 patients	131 (110-164)	1.54 (1-2)	110 (60-162)	0.45 (0.2-1.0)	13 (7-20)
<i>Group 2</i> Response to chlorpropamide only: 10 patients	250 (200-380)	1.60 (1-3)	123 (80-160)	0.57 (0.2-1.0)	18 (17-20)
<i>Group 3</i> Unresponsive: 5 patients	243 (215-270)	1.50 (1-2)	229 (215-270)	0.45 (0.2-0.5)	22 (16-29)

shorter duration of the diabetic state than those who did not respond. These differences between the groups were slight and statistically insignificant; however, the mean pretreatment daily insulin dose was 11 U. in the responsive group and 25 U. in the nonresponsive group, and only 1 of the 40 patients in the former group gave a history of past ketoacidosis, while 4 of 14 in the latter group had such a history.

Twenty-eight patients who had taken tolbutamide were subjected to a trial of chlorpropamide in order that blood sugar responses and magnitudes of dosage of the 2 drugs might be compared (TABLE 4). Chlorpropamide in daily doses of 0.2 to 1.0 gm. was substituted for tolbutamide in 13 patients (Group 1) who previously had responded in an optimal fashion to the latter drug. The degree of control with chlorpropamide in a mean dose of 0.45 gm. daily compared favorably to that obtained with the daily administration of 1.54 gm. of tolbutamide, and no patient who previously had been controlled

with tolbutamide was found to be resistant to chlorpropamide. Serum levels of chlorpropamide averaged 13 mg. per cent in this group.

Fifteen additional patients had been taking 1.0 to 3.0 gm. of tolbutamide daily, but displayed fasting blood sugar levels of 200 to 380 mg. per cent and could be classified as relatively resistant to tolbutamide at this dosage level. When chlorpropamide was substituted, 10 of these individuals (Group 2) showed a fall in mean fasting blood sugar levels from 250 to 123 mg. per cent, and each met our criteria for response. At the time of the favorable response, the dose of chlorpropamide ranged from 0.2 to 1.0 gm. daily, and serum levels of the drug averaged 18 mg. per cent. However, in 5 subjects (Group 3) the fasting blood sugar levels fell only slightly, from a mean of 243 to 229 mg.

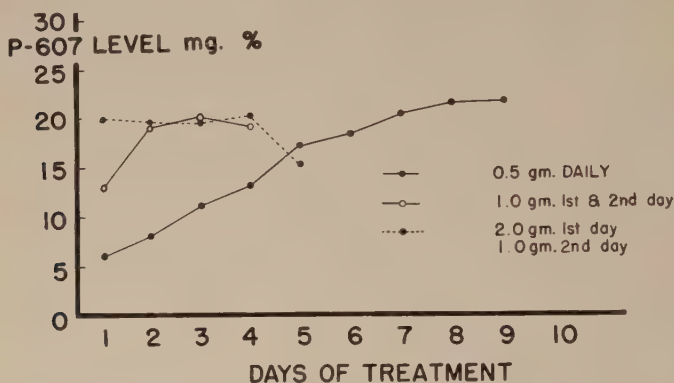


FIGURE 1. Effect of initial chlorpropamide dosage on chlorpropamide blood levels. Solid curve with closed circles represents data from 4 patients treated with dosages shown; the solid curve with open circles, 2 patients; and the broken curve, 2 patients.

per cent as chlorpropamide in daily doses of 0.25 to 0.50 gm. was substituted for an average dose of 1.5 gm. of tolbutamide. Serum levels of chlorpropamide ranged from 16 to 29 mg. per cent in these unresponsive patients.

It became apparent early in the study that some correlation existed between magnitude of dosage, serum levels of the drug, and response. Accordingly, initial daily doses ranging from 0.5 to 2.0 gm. were administered to small groups of subjects, and the relationship between the serum levels obtained and the lapse of time needed for attainment of satisfactory blood sugar levels was noted (FIGURE 1). When a dosage schedule of 0.5 gm. daily was employed, about 4 days were necessary to achieve those serum concentrations of the drug that were found to be optimally effective. This dose resulted in progressive elevation of serum levels for 9 to 10 days, after which constant levels were maintained. When larger initial doses were used, higher serum levels were achieved much more rapidly. With either method, optimal control of hyperglycemia was attained only after 4 to 12 days (average 6 days).

Blood levels of chlorpropamide obtained at random in a group of 20 patients maintained for 1 to 20 weeks on a daily dose of 0.5 gm. were found to range from 16 to 20 mg. per cent (FIGURE 2). In patients in whom the drug was

discontinued, serum levels as high as 5 mg. per cent persisted as long as 72 hours.

Endocrine function. The effect of chlorpropamide upon thyroid function was assayed by determination of serum protein-bound iodine (PBI) levels,

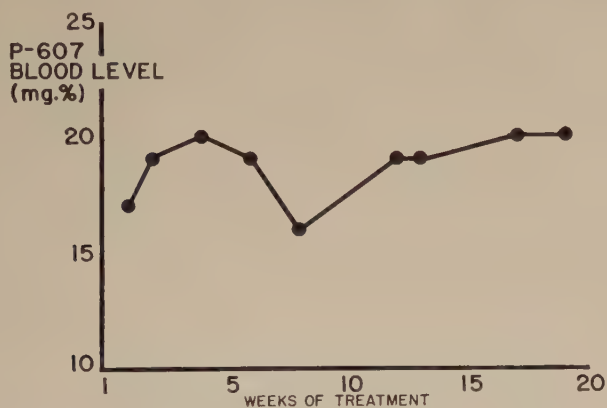


FIGURE 2. Averages of serial chlorpropamide blood levels of 20 patients maintained on 0.5 gm. daily.

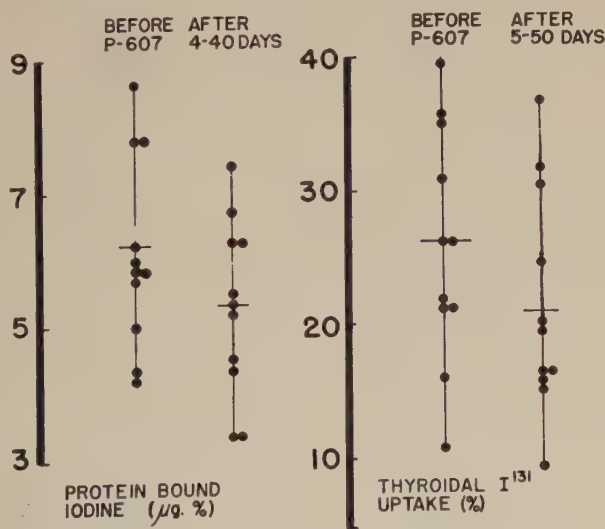


FIGURE 3. Effect of chlorpropamide on thyroid function tests.

24-hour thyroidal radioiodine uptakes, and serum cholesterol values, both during the pretreatment period and after 4 to 50 days of therapy and, subsequently, at random intervals. Comparison of these values suggests some inhibition of thyroid function, as the mean PBI fell from 6.2 to a level of 5.4 µg. per cent, and the mean uptake fell from 27 to a value of 22 per cent (FIGURE 3). This effect of a sulfonylurea derivative has been described by

McGavack.¹ No clinical evidence of hypothyroidism or enlargement of the thyroid gland has been observed to date. Cholesterol values in 21 subjects fell from a mean of 264 to 232 mg. per cent. This response probably reflects an improvement in diabetic control rather than an alteration in thyroid function although a direct effect on lipid metabolism cannot be excluded. In 2 patients with concomitant thyrotoxicosis and hyperglycemia, chlorpropamide proved to be an effective hypoglycemic agent.

The relationship of chlorpropamide therapy to adrenal cortical function was studied by quantitative measurement of daily 24-hour urinary excretion

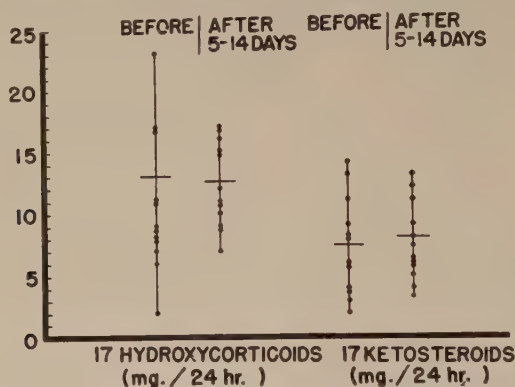


FIGURE 4. Effect of chlorpropamide on urinary steroid excretion.

of 17 hydroxycorticoids and 17 ketosteroids. Values for each patient were averaged individually, and control levels were compared with those obtained during treatment. No changes attributable to therapy were noted. Corticoids averaged 13.5 mg. per day before therapy and 13.0 mg. per day during therapy; ketosteroids averaged 8.0 mg. per day in the control period and 8.5 mg. per day during treatment (FIGURE 4). Each of 3 subjects with Cushing's syndrome due, respectively, to adrenal cortical hyperplasia, adrenal cortical adenoma, and exogenous administration of 6 methyl prednisolone, displayed significant lowering of blood sugar and became normoglycemic with chlorpropamide treatment. This response differs from that reported by others who failed to observe such an effect when using tolbutamide.²

A 25-year-old juvenile diabetic who was also treated had undergone a surgical hypophysectomy in the hope that his severe progressive retinitis proliferans might be controlled. Hypophysectomy resulted in a decrease of his daily insulin requirement from 80 to 14 U. of NPH. The daily administration of 2.0 gm. of chlorpropamide, in addition to his usual insulin dose, failed to alter either blood sugar levels or insulin requirement. An evaluation of the effects of chlorpropamide on gonadal function was not made.

Reactions to chlorpropamide. Numerous tests were done to assay the influence of chlorpropamide on the functions of the liver, kidney, and bone marrow (TABLE 5). Such determinations were made prior to the inception of chlorpropamide therapy and were repeated after 5 to 10 days and, later

at random intervals throughout the period of study. Averages and ranges of values obtained before therapy were compared to the mean determinations made during treatment.

No significant alterations in liver function were evident from the comparison of values of thymol turbidity, serum alkaline phosphatase, serum

TABLE 5
TESTS OF LIVER, KIDNEY, AND BONE MARROW FUNCTION

Test	No. of patients	Values before chlorpropamide	Values after chlorpropamide
Thymol turbidity (units)	16	7.5 (0-20)	9.4 (0-40)
Serum alkaline phosphatase (SJR units)	13	6.8 (4-14)	6.3 (4-16)
Serum bilirubin (direct/indirect, mg. %)	14	0.3/0.3 (0.1/0.1-0.6/0.2)	0.2/0.1 (0.1/0.1-0.5/0.4)
Prothrombin concentration (%)	9	83 (52-100)	81 (42-100)
Albumin/globulin (gm. %)	9	4.3/3.3 (2.6/1.7-4.8/4.4)	4.1/3.5 (2.5/2.6-4.6/4.0)
Bromsulphalein retention (percentage after 45 min.)	10	3.5 (0-14)	2.5 (0-8)
Cephalin flocculation (units)	10	trace (0-2+)	trace (0-1+)
Serum glutamic oxaloacetic transaminase (units)	10	19.7 (11-29)	29.8 (7-103)
Serum creatinine (mg. %)	9	1.4 (1.0-1.7)	1.4 (1.0-1.9)
Blood urea nitrogen (mg. %)	34	16 (8-31)	18 (8-32)
Serum uric acid (mg. %)	9	3.6 (3.0-4.4)	3.7 (3.0-4.7)
White blood cell count (per cu. mm.)	25	7400 (4,000-20,000)	6400 (3,500-10,400)
Neutrophils (percentage)	24	61 (43-77)	60 (35-74)
Hemoglobin (gm. %)	21	13.9 (12-16)	13.4 (11-16)

bilirubin, prothrombin concentration, serum albumin and globulin, Bromsulphalein retention, cephalin flocculation, and serum glutamic oxaloacetic transaminase levels. Similar negative results were found when renal function was investigated by measurement of serum creatinine, blood urea nitrogen, and uric acid values, and when bone marrow function was appraised by comparing white blood cell and differential counts and hemoglobin concentrations.

Untoward reactions to chlorpropamide could be grouped roughly into 4 categories. Of the 54 patients to whom the drug was given, 9 (17 per cent) manifested undesirable effects. Three individuals, all of whom were lean, mild diabetics, experienced classic hypoglycemic responses. These episodes

occurred in both the late postprandial and fasting states and were rapidly controlled by glucose administration. Decrease in the maintenance dose abolished these reactions. Two subjects complained of burning midepigastric distress following ingestion of the tablets. Each had registered similar complaints before being placed on therapy, but felt that the drug exaggerated the discomfort. This complaint was easily controlled by administering antacids with the drug or by giving the drug with meals. Many patients with varying degrees of severe cerebral arteriosclerosis were treated with chlorpropamide. In 2 of these, cerebral vascular accidents were observed during treatment. In another, episodes of accentuated mental confusion occurred. In none was actual hypoglycemia documented, although this would appear to be the most likely explanation for their symptoms.

For the most part, no alteration of hepatic function could be attributed to the drug. However, in one patient with biopsy-proved hepatic cirrhosis, slight enlargement of the liver and increase in thymol turbidity titers occurred during chlorpropamide therapy. Similar episodes had occurred during periods when the drug was not being given. There were no manifestations of dermal hypersensitivity to the drug.

Summary and Conclusions

Chlorpropamide was given to 54 selected diabetic patients, and an adequate response was obtained in 40 of these. As a group, the patients tended to be mild diabetics requiring little or no insulin, were obese, and middle aged or older.

Ten of 15 patients unresponsive to tolbutamide responded optimally to chlorpropamide. No patient responsive to tolbutamide failed to respond to chlorpropamide.

The effective maintenance dose ranged from 0.2 to 1.0 gm. daily and resulted in serum levels of 13 to 20 mg. per cent. A priming dose raised the serum levels more rapidly, but failed to hasten the hypoglycemic response.

Slight depression of thyroid function, as determined by the usual tests, was observed, but no clinical manifestations of thyroid deficiency or enlargement of the thyroid gland occurred.

No alterations of adrenal cortical, renal, hematopoietic, or cardiac function were observed. Liver function remained normal except in 1 patient with known hepatic cirrhosis.

Untoward reactions occurred in 9 of 54 patients (17 per cent). Of these, 5 had reactions consisting of hypoglycemia or epigastric distress, which were readily controlled. Three of the patients with cerebral arteriosclerosis deteriorated during therapy.

Two patients with thyrotoxicosis and 3 with Cushing's syndrome associated with hyperglycemia became normoglycemic with chlorpropamide treatment.

It would seem that chlorpropamide is a more potent hypoglycemic agent than tolbutamide and, as a consequence, is more likely to produce symptomatic hypoglycemia and its undesirable consequences.

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Discussion of the Paper

CARLOS P. LAMAR (*Miami, Fla.*): I propose to make a short comment on another aspect of the side effects of sulfonylureas that I do not believe has been mentioned in these pages.

Hamwi and others reported on the number of patients who, showing late-developing lack of response or resistance to sulfonylureas, were necessarily returned to insulin therapy. We have 7 patients who were originally selected as ideally suited for this type of therapy and who apparently responded well for periods of 2 to 7 months, after which it was necessary to return them to insulin. The original daily doses of insulin required for maintenance of adequate diabetic control for periods of several years had ranged between 20 and 30 U. Following the use of tolbutamide and carbutamide in this group, the amount of insulin required to obtain adequate diabetic control ranged from 60 to 90 U., or 3 times the pretolbutamide or carbutamide dose level. These individuals have been observed now for periods of 6 months to almost 2 years and, although their insulin requirements are declining gradually, they still require 100 per cent more insulin than they did before.

With respect to Hamwi's cases of cerebral arteriosclerosis with manifestations of clinical hypoglycemia, in which he was unable to substantiate the presence of "chemical" hypoglycemia, I suggest a very simple therapeutic test that we have used very successfully for quite some time: administer sugar intravenously, even if the individual has a blood sugar level of 250 to 300 mg. per cent or more. These figures do not mean that that individual, who probably has a decreased transfer or osmosis through the cerebral cell membrane, is not hypoglycemic; the proof of hypoglycemia lies in the fact that the administration of glucose under these circumstances is followed by a dramatic correction of the clinical hypoglycemic symptoms. An adjustment in the treatment of these individuals to a less rigid adherence to the concept of so-called normal glycemic levels would help them and, perhaps, might be a factor in the prevention of those cerebral vascular accidents mentioned as of obscure origin in this and other individuals.

CLINICAL EXPERIENCE IN THE USE OF CHLORPROPAMIDE IN THE TREATMENT OF DIABETES MELLITUS*

J. A. García Reyes, J. C. Peña, E. Barroso, F. B. S. Anton, C. Gonzalez
Metabolic-Endocrine Division, National Institute of Nutrition, Mexico, D.F., Mexico

It is our purpose in this paper to describe our clinical experience with chlorpropamide in the management of the adult and juvenile diabetic patient.

In fifty unselected subjects from the Diabetic Clinic of the Nutritional Disease Hospital in Mexico, D.F., Mexico, the influence of chlorpropamide has been studied with particular reference to changes in hyperglycemia and the general health of the patient. In a few cases, studies of thyroid function were carried out.

Controls

The hypoglycemic effect of the compound was first tested in 10 healthy normal young adults who were used as a control group. These patients were given glucose tolerance tests using a standard 100-gm. oral dose, and measuring blood sugar every hour for 5 hours. This study was repeated on the same subjects 3 to 6 days later, each patient receiving a test dose of chlorpropamide (1 gm.) 1 hour before the glucose ingestion.

Results. The results of this study are shown in FIGURE 1. There is a definite flattening of the curve after chlorpropamide. It is interesting to note the significant depression in the fasting blood sugar values during the test in 6 of the 10 subjects.

Diabetic Patients

Material and methods. In this group of 50 cases, diabetes was of the adult or "stable" type in 45 and "juvenile" or "brittle" in 5. The duration of the disease ranged from 3 months to 19 years, with an average of 6½ years.

Most patients were between 40 and 50 years old, the ages ranging from 16 to 70 years. The age distribution is shown in TABLE 1. Forty-four cases had vascular manifestations of diabetes; among the 50 cases there were 30 other complications, as shown in TABLE 2. Before the beginning of the study, 8 patients were on diet alone, 33 were receiving insulin, 5 were on carbutamide or tolbutamide, and 4 were receiving no treatment (TABLE 3).

All patients were kept on a constant calculated diet during a control period and throughout the study. The diet components were measured in terms of familiar household serving practices (ambulatory cases), or were supplied by the dietetic kitchen (hospitalized cases).

Chlorpropamide therapy was usually started in divided doses of 750 mg. to 1 gm. in 24 hours. The duration of treatment was 8 to 69 days.

Patients who had failed to respond to diet alone or who were on other oral hypoglycemic agents received chlorpropamide as the only medication. The dose of the drug was modified subsequently in each case, according to

* The work reported in this paper was aided by a grant from Pfizer de Mexico, Mexico, D.F., Mexico, who supplied the chlorpropamide used.

the initial response. Chlorpropamide was given alone when daily insulin requirements were 20 U. or less.

A reduction of 75 per cent of the insulin dose was made in patients receiving more than 20 U. per day, and chlorpropamide was given in the same fashion

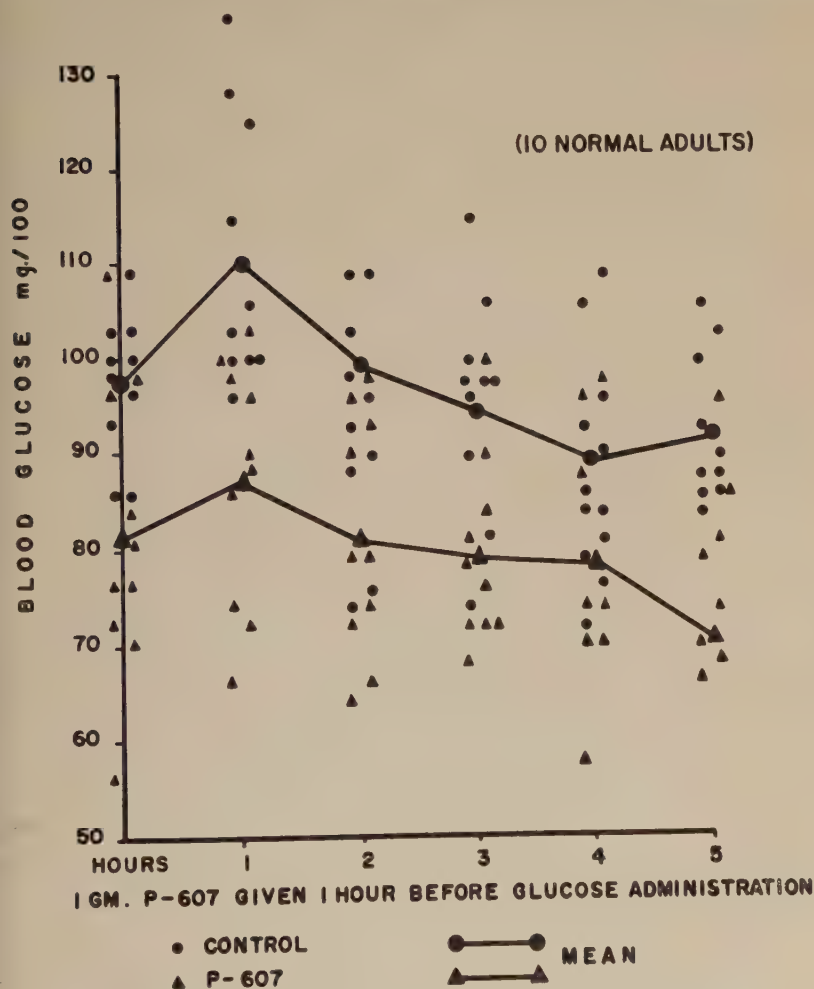


FIGURE 1. Changes in glucose tolerance test with chlorpropamide.

described for the whole group. Further reductions in insulin were made according to the response.

Diabetic control was evaluated on the basis of glycosuria and glycemia. Urine sugar was checked three times a day by the qualitative Benedict's method, and the results were recorded in the usual way from 0 to ++++. Blood glucose determinations were made by the Folin-Wu method in the fasting state and, occasionally, after meals in the ambulatory cases.

TABLE 1
AGE DISTRIBUTION OF PATIENTS

Age (years)	No. of cases
10-19	2
20-29	3
30-39	2
40-49	15
50-59	21
60-69	5
70-79	2

TABLE 2
VASCULAR MANIFESTATIONS AND COMPLICATING DISEASES

Disorder	No. of cases
Retinopathy.....	27
Nephropathy.....	5
Cataract.....	7
Neuropathy.....	18
Urinary infection.....	3
Gangrene.....	1
Arteriosclerotic heart disease.....	11
Tuberculosis.....	2 (1 inactive)
None.....	14

TABLE 3
DATA REGARDING PREVIOUS TREATMENT

Treatment	No. of cases
Diet alone.....	8
Insulin.....	33
Tolbutamide.....	4
Carbutamide.....	1
None.....	4

Outpatient cases were followed twice weekly and close clinical observation was maintained. Particular emphasis was placed on the detection of toxic manifestations. In twenty-seven cases, liver function tests were done prior to the treatment and were repeated about three weeks later. These included the Bromsulphalein test and measurements of serum bilirubin, cephalin cholesterol flocculation, and thymol turbidity. Complete blood counts were done before chlorpropamide in forty cases and repeated toward the end

of the therapeutic trial. Because of the possibility of crystalluria, urinalysis was done in all cases.

Results

According to the clinical and laboratory data observed during chlorpropamide treatment the diabetic patients were placed in the following groups (TABLE 4):

TABLE 4
CRITERIA FOR CLASSIFICATION OF RESPONSE TO TREATMENT

Classification	Characteristics
Good	At least three of the following: a) Fasting blood sugar less than 130 mg. b) Postprandial blood sugar less than 170 mg. c) Zero to 1 plus glycosuria. d) Complete or almost complete (75 per cent) reduction in insulin requirement.
Fair	a) Fasting blood sugar less than 150 mg. b) Postprandial blood sugar less than 200 mg. c) One plus to 2 plus glycosuria. d) At least 50 per cent reduction in insulin requirement.
Poor	All others.

Good-response group. Constituting 56 per cent of the total, patients in this group display at least 3 of the following characteristics: (1) a fasting blood sugar of less than 130 mg. per cent; (2) a postprandial blood sugar less than 170 mg. per cent; (3) zero to 1 plus glycosuria; and (4) complete or almost complete substitution (75 per cent reduction) in insulin requirements.

Fair-response group. Patients (22 per cent of total) in this group show (1) a fasting blood sugar of less than 150 mg. per cent; (2) postprandial blood sugar of less than 200 mg. per cent; (3) one plus to 2 plus glycosuria, and (4) at least 50 per cent reduction in insulin requirements.

Poor-response group. This group is constituted of all patients (22 per cent) not classified in the other groups.

Using the above criteria, which are related entirely to the control of the hyperglycemic and glycosuric aspects of diabetes, a good response was observed in 28 of 50 cases (56 per cent), a partial effect in 11 (22 per cent), and none in 11 (22 per cent).

Discussion

Of the 28 patients in the good-response group, 16 were on insulin treatment at doses ranging from 10 to 60 U. per day, with a mean of 28 U. A complete substitution of insulin by chlorpropamide was obtained in 15 patients and an almost complete substitution (75 per cent) in 1.

Two patients were on tolbutamide and 1 on carbutamide. There was a

transitory period of hyperglycemia and intense glycosuria immediately following the substitution of tolbutamide for chlorpropamide in 1 case, but glucose levels promptly returned to normal within the following 48 hours.

The nine remaining patients were without insulin or other hypoglycemic agents at the start of the study. They failed to respond to diet alone, but responded to chlorpropamide.

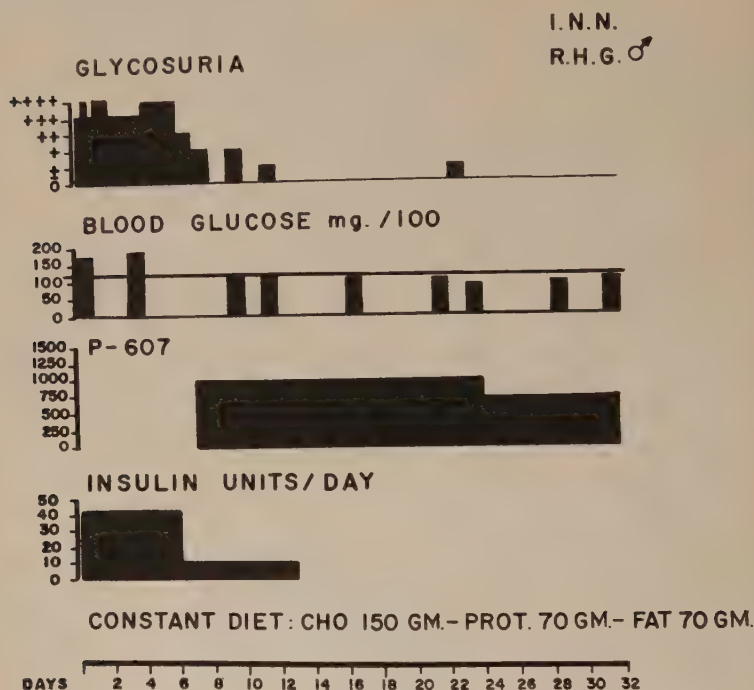


FIGURE 2

Fasting blood sugar values in the pretreatment period in the whole group ranged from 90 to 350 mg./100 cc., the average being 203 mg. After chlorpropamide administration, values varied from 79 to 161, the average being 110 mg./100 cc.

The favorable response in the cases considered "good" occurred between the second and eighteenth days, the average at 5.5 days. In most patients, after controlling their diabetes, the average daily maintenance dose of chlorpropamide was 500 mg.

An illustrative case is presented in FIGURE 2. The drug was well tolerated, but we noted slight hypoglycemia in three cases.

Case 44 seemed to be a relative failure at the beginning, as there was a period of 17 days before glucose levels were controlled with 1 gm. of chlorpropamide and decreasing doses of insulin. Ten days later we were able to discontinue insulin and to control the patient with chlorpropamide (FIGURE 3.)

In the fair-response group, 7 patients were receiving insulin in doses that ranged from 20 to 80 U. per day, the average being 43 U.—that is, 15 U. more than the mean of the good-response group. At the beginning of the study patient was on tolbutamide, 2 were on diet alone (but their diabetes was uncontrolled), and 1 was receiving no treatment. Chlorpropamide administration was maintained for an average of 22 days, with a range of 8 to 38 days. The mean daily maintenance dose in this group was 770 mg.

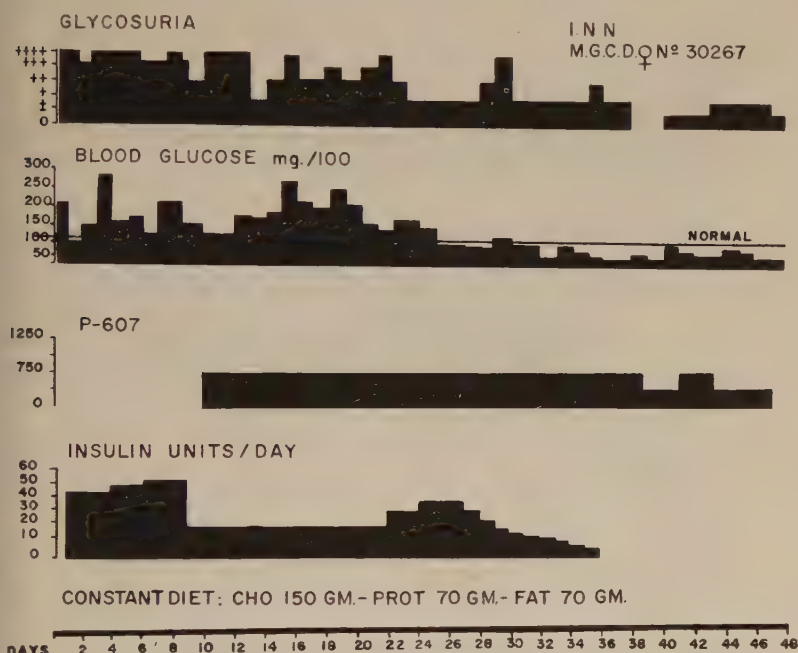


FIGURE 3

Average values for the fasting blood sugar dropped from 207 mg./100 cc. to 152 mg./100 cc. after chlorpropamide administration. A typical case is presented in FIGURE 4.

At the beginning of the study, 10 patients in the poor response group were receiving 25 to 60 U. of insulin per day, the average being 37 U., and 1 was on tolbutamide but had not responded to this compound. Our five patients with juvenile diabetes are included in this group. The 6 remaining patients had the stable type of the disease.

No significant fall in hyperglycemia was observed, and a reduction in the insulin requirements of only 25 to 50 per cent was obtained in 3 cases. Chlorpropamide was given in a dose of 1 gm. per day; the average duration of treatment was 12.6 days. A case is presented in FIGURE 5.

Also studied was the possibility of a correlation between the response to chlorpropamide and several factors such as the type of diabetes, the presence

of obesity, the previous insulin requirements, and the duration of the disease. The results are shown in FIGURE 6.

The only positive correlation found was that between response and disease type, as no single case of juvenile diabetes responded to chlorpropamide treatment.

Side effects and toxic manifestations. Eleven patients complained of somnolence, weakness, and a "dizzy feeling" that were thought to be due to

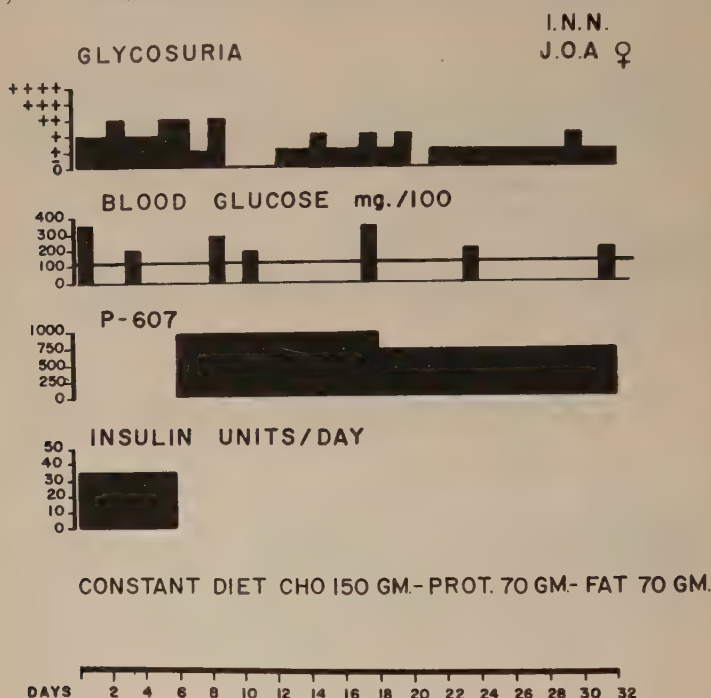


FIGURE 4

hypoglycemia, but glucose determinations failed to confirm this impression. Dermatological lesions were seen in 3; of these, 1 was an exfoliative dermatitis that promptly subsided after discontinuance of therapy. The other 2 patients had urticaria; this disappeared in 1 in spite of continuing treatment and, in the remaining case, after reducing the dose from 1 gm. to 750 mg. per day. Four patients complained of constipation.

No change in the liver function tests was shown in the 27 patients in whom such studies were done before and during chlorpropamide therapy.

Aside from transitory reduction in granulocytes in 3 cases, no toxic effects on the hematopoietic system were observed.

No crystalluria was found during the period of observation.

Thyroid function, as demonstrated by uptake of I^{131} by the thyroid gland, was measured in 7 cases before the onset of chlorpropamide treatment; this was repeated 2 to 3 weeks later; no significant depression was observed.

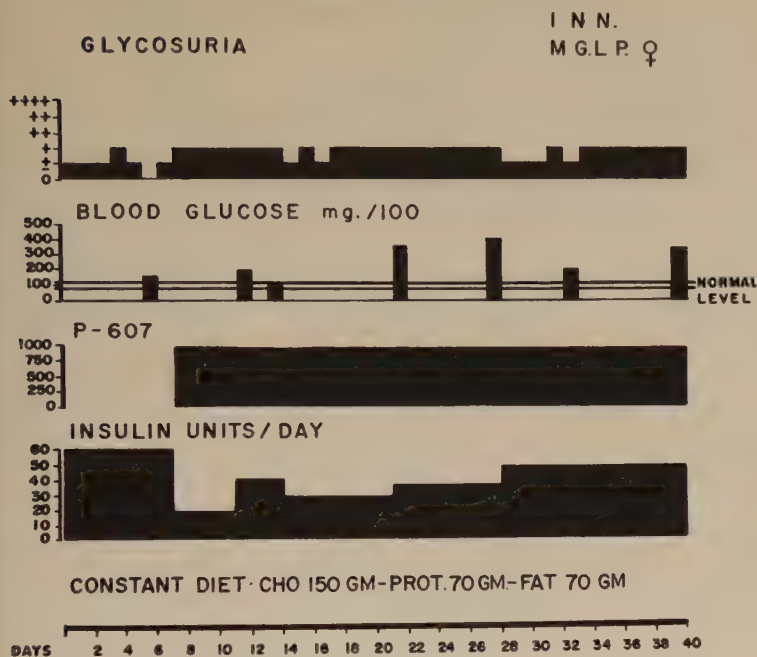


FIGURE 5

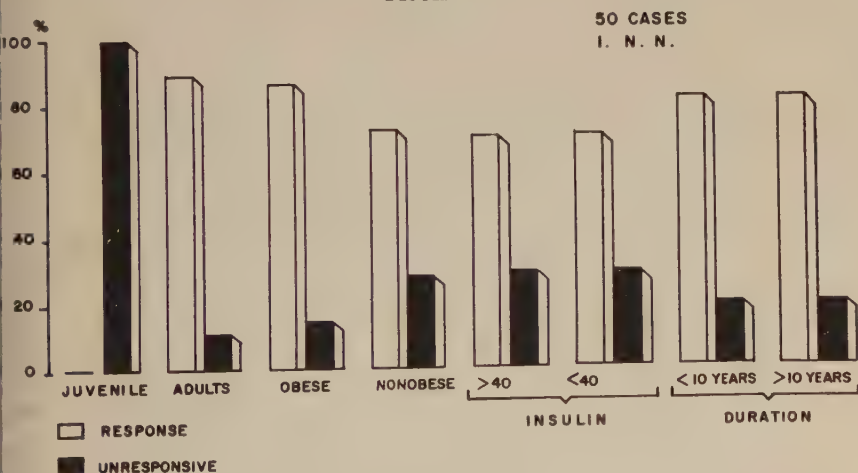


FIGURE 6. Response of diabetic patients to chlorpropamide.

Summary and Conclusions

Chlorpropamide was administered to ten normal healthy adults and fifty diabetic patients, in order to study its hypoglycemic activity; a flattening of the glucose tolerance curve was seen after its administration.

Fifty-six per cent of the diabetic patients responded favorably to the

treatment, and a complete substitution of the drug for insulin was obtained in all but one patient in this group.

No response was obtained in the juvenile diabetic patients.

No alterations in liver function or in the hematopoietic system were observed.

Side effects included somnolence, weakness, and dizziness in eleven cases.

Response does not seem to be related to the duration of the diabetes, to the insulin requirements (within certain limits), or to deviations from normal weight.

SUMMARY OF THE MONOGRAPH

Charles H. Best

Charles H. Best Institute, Toronto, Ont., Canada

We all appreciate the fact that new evidence derived from the use of oral hypoglycemic agents has focused a searching light on the formation, liberation, and action of insulin on a variety of metabolic processes and on diabetes and its treatment. We acknowledge again our debt to Loubatières¹ for his pioneering work and our indebtedness to our German colleagues²⁻⁴ for their great and essential chemical and clinical contributions to this rapidly expanding field. I was just beginning to take a scientific interest in metabolism when the work on the hypoglycemic effect of guanidine was published in 1918.⁵ We lived through the studies and clinical use of Synthalin,⁶ Myrtilin,⁷ and many other disappointing hypoglycemic agents.⁸ However, there is no doubt, as we all realize, that a new era of oral hypoglycemic therapy is here. There is every indication that, if these drugs are carefully used, a great deal of comfort and help can be given to many properly selected patients. It is fortunate that a large number of experienced people have contributed to this monograph in appraising the value of chlorpropamide.

There are a few papers that relate to insulin alone; this raises the total to fifty thousand, plus a few, since 1921. Robert Williams* has given us a comprehensive discussion of the destruction of insulin by homogenates and fractions of many tissues. The activity in tissues varies from the maximum in liver to the minimum in blood plasma. Williams makes the distinction between the reductive and proteolytic mechanisms of degradation (glucagon is destroyed only proteolytically). Little change in the insulin-destroying activity was noted after infective and traumatic insults. Traumatization of rat muscle *in vivo* did increase the insulin-destroying effect. Williams paid eloquent tribute to Mirsky's* original contributions in this field. In discussion, Mirsky emphasized that, in referring to insulinase, he envisaged a labile saturated proteolytic system. Both Williams and his colleagues and Mirsky have now prepared a highly purified insulinase, and it is obvious that studies of the physiological role of this enzyme will be greatly facilitated.

Prout and Evans* have measured the *in vivo* disappearance of I¹³¹ insulin and feel that the fixation of the hormone by tissues is not a significant factor in this removal. The intact insulin is distributed in a volume similar to that of extracellular water; that is, it is distributed throughout the extracellular space.

There are many problems on which we all seek further information. What is the mechanism of action of any of the oral hypoglycemic agents? What evidence is there that an increased secretion or liberation of insulin is produced? Loubatières provided our first evidence in a classically physiological way, that is, by removing the pancreas and noting the disappearance of the effect. Houssay⁹ and many other authors have confirmed this. Most of

* Elsewhere in this monograph.

the recent data have been obtained in studies on tolbutamide, and the situation is still unsettled. I shall not attempt to review here all the direct and indirect evidence that has been obtained in a variety of ways. There have been studies on the morphological changes of the beta cells¹⁰⁻¹⁷ and on the amounts of insulin extractable from the pancreas.¹²⁻¹⁸ The direct attack—that is, estimation of the insulin content of plasma—has many supporters.¹⁹⁻²⁵ Indirect approaches have utilized cross-circulation tests (Loubatières,²⁶ Fôa,²⁷ Colwell²⁸⁻²⁹), perfusion experiments, eviscerated preparations,³⁰⁻³² and a great variety of metabolic studies—glucose tolerance tests³³⁻³⁷ and estimations of such factors as serum inorganic phosphate,^{34, 36, 38-40} potassium,³⁷⁻⁴⁰ nitrogen balance,³⁷⁻⁴¹ blood pyruvate^{36, 37, 42-44} and lactate,^{36, 42-44} plasma UFA,²²⁻⁴⁵ ketones,^{22, 42} and the RQ.^{22, 41, 46} The effect on the decrease in sugar output by the liver^{34, 47-49} sometimes has been used, in effect, as a test for insulin, and changes in enzyme activity in the liver obviously might be similarly interpreted⁵⁰ in certain cases.

The importance of knowing the extent and duration of insulin liberation produced by the various oral hypoglycemic agents cannot be overemphasized. How can we know to what extent insulin itself changes enzyme activity in the liver and how much direct action a drug is exerting without this information? The valuable studies of Sass and Handelsman* on transaminases in liver homogenates of animals that have received chlorpropamide illustrate this point. Indirect approaches can help, of course. The situation is similar when we consider sugar production by the liver, as I shall point out later.

I shall not attempt to give an appraisal of all the indirect evidence that is very strongly in favor of some insulin liberation after administration of the oral agents. Positive direct evidence has been reported by von Holt,¹⁹ Goetz,²² and Pfeiffer *et al.*²⁵ The methods used are not above criticism, by the investigators themselves as well as by others. Recently, Renold (personal communication) and his group were unable, even with their very sensitive and reliable method, to detect in man any increase in plasma insulinlike substances after administration of the oral agents. They do find an increase in the amount of these materials in pancreatic vein blood of the dog. A survey of this field leads to the conclusion that one aspect of the action of the hypoglycemic oral agents is almost certainly the liberation of a certain amount of extra insulin, but the possibility remains that the mere presence of a little insulin furnishes the essential link.

With respect to the action of insulin on the liver, I believe Miller rightly emphasized that it will be necessary to understand completely the physiological action of insulin before we can hope to comprehend the pharmacological action of the oral hypoglycemic agents.

Craig, Drucker, Miller, and Woodward* showed that small doses of insulin in human subjects cause a decrease in splanchnic output of glucose without evidence of increased peripheral use. Tolbutamide produces the same effect and, therefore, *may* act by liberating small amounts of insulin.

Weinhouse's* studies on the effect of small amounts of insulin on the curve of disappearance of C¹⁴ glucose in human subjects and in dogs have sup-

* Elsewhere in this monograph.

ported this conclusion. In human subjects he found that a slow infusion of insulin produced a decreased hepatic output of glucose, but no indication of increased peripheral use. Larger doses of insulin gave both effects.

Madison, Unger, and Kaplan* found in human subjects that slow infusions of glucagon-free insulin, which produced a good fall in arterial blood sugar, caused no change in arteriovenous difference. Tolbutamide produced the same effect. They reported that in dogs with portacaval fistulas the administration of insulin consistently produced a decreased hepatic output of sugar. These workers emphasized the various differences between endogenous and exogenous insulin. The only established difference between natural and exogenous insulin relates to the route of entry of the hormone. We all know that depancreatized dogs and some severe diabetic patients have lived a lifetime (that is, six years and thirty-seven years, respectively) on exogenous insulin.

Schambye and Tarding* found that tolbutamide produced an inhibition of hepatic glucose output as judged by plateauing of the curve of specific activity of C^{14} glucose in normal and depancreatized dogs and in diabetic patients. The inhibition in the normal organism lasted no longer than 40 min., but in the diabetic it lasted at least 2 hours. Although insulin given intravenously and intraportally in normal dogs could not be found to decrease hepatic output of sugar, tolbutamide did produce this effect. These differences in the observed effects of insulin emphasized again the necessity of attempting to standardize our tools—the type and amount of insulin, for example, and many other variables.

I propose now to emphasize a point or two on the effect of insulin on the liver. Perfusion of the isolated liver (Miller, Hamff*) has demonstrated a rather prompt effect of insulin on fat formation, and I shall not be surprised to see revealed a prompt, direct action on sugar and amino acids in this issue. The fundamental interest and practical importance of this subject must be obvious. We cannot readily investigate the *direct* action of the oral agents on liver if these agents liberate insulin which, in turn, affects the liver. There is, of course, no doubt about many very important delayed and perhaps indirect actions of insulin on the liver. De Duve⁵¹ and his colleagues recently discussed this matter at the International Diabetes Federation in Düsseldorf, Germany. While I feel that small amounts of insulin are probably liberated or, at least, are necessary for the action of the oral agents, I must stress the fact that the exploration of the direct effect of these agents on the liver has hardly been started. Downie* has aroused our interest by reference to Bornstein's⁵² work on the effect of chlorpropamide on the oxidation of C^{14} -labeled alanine to CO_2 in liver slices. Mirsky* has stimulated us again with his finding that tolbutamide prevents the diabetogenic action of somatotropin in dogs. Zarowitz* compared the glucose and tolbutamide tolerance tests in nondiabetic and diabetic patients. He feels that the tolbutamide test detects some mild diabetics that the older test would not reveal. Stolstoi* did not see the advantage of and justification for the tolbutamide test. However, we are concerned particularly in this monograph with chlor-

* Elsewhere in this monograph.

propamide. It is obvious that this compound is an effective agent and that, under certain circumstances, it can be given safely for prolonged periods to humans.

McLamore's work* gives us a great deal of information on the relation of chemical structure to hypoglycemic action. Schneider† and Mary Root,‡ agreed on the greater activity of chlorpropamide as compared to tolbutamide in mice, rats, and dogs. The former was slightly more toxic. Chlorpropamide was active in monkeys.

In rats there was a degranulation of beta cells; no effect in alloxan diabetes; a positive effect in hepatectomized preparations; and an enhanced effect in adrenalectomized animals. Schneider reported certain toxic effects (predominately on the CNS) with large doses of chlorpropamide in dogs, but he stated that 120 mg./kg. was well tolerated for 20 months. There was no change in various liver function tests; this is in contrast to the findings of A. Sirek and O. Sirek⁵³ in our laboratory with tolbutamide which, at a level of 30 mg./kg., does produce hepatic injury in normal and diabetic dogs. We should test chlorpropamide under the same conditions, using dosages that produce the same hypoglycemic effects. The liver lesions seen in a few patients receiving chlorpropamide resemble strikingly those seen in dogs given tolbutamide, but the finding in dogs must not be "transferred" to human subjects; the striking absence of liver lesions in many, many thousands of patients receiving tolbutamide must be emphasized again.

Lundbaeck* and Clarke* differ in their finding of glucose uptake by diaphragm in *in vitro* studies. Lundbaeck has reported an increased carbohydrate tolerance in patients given chlorpropamide or tolbutamide. Danowski* has studied chlorpropamide in carefully standardized normal and diabetic subjects. He feels that the first effect may be to decrease gluconeogenesis; the second, perhaps, to increase insulin liberation. He studied particularly blood glucose and phosphate, detecting no change in serum inorganic phosphate in diabetics given chlorpropamide. His report on the increased immediate utilization of sugar and the fall in serum phosphate after cortisone is intensely stimulating.

On the clinical use of chlorpropamide, all agree, as Tolstoi summarized, that chlorpropamide is an effective agent, and one more potent than tolbutamide. As Beaser emphasized, the drug should be criticized only for its production of side effects when used at the minimal effective dose.

Conn* repeatedly stressed the close relationship between blood level and effect: at the same blood levels of tolbutamide and chlorpropamide the same effects were produced. Craig* agreed, but others have been less certain of the significance of this relationship. Kelly West* feels that chlorpropamide is more potent intrinsically, but he has not as yet measured blood levels. Beaser, using different doses of chlorpropamide, found a direct relationship between dose and blood level, but not necessarily between dose and blood sugar values. Hamff* reported three cases of jaundice with the use of large

* Elsewhere in this monograph.

† Of Chas. Pfizer & Co., Inc., Brooklyn, N. Y.

‡ Of Eli Lilly and Company, Indianapolis, Ind.

doses of chlorpropamide. Two of these patients later did well on smaller doses and the third was transferred to tolbutamide.

There have been primary and secondary failures with both drugs and, in certain cases, the other drug was effective. Nabarro's* statement on the incidence of acquired resistance to tolbutamide was noteworthy, as many as 14 per cent of his cases showing resistance in 18 months. Insulin consistently has been found to be effective, and its dose can safely be increased when necessary.

The selection of cases for oral therapy is a vitally important matter. Duncan,* Hamwi,* and many others have discussed this problem. Ketosis on withdrawal of insulin is considered to contraindicate the use of the oral agents. In addition to careful selection of patients, Duncan emphasized that these drugs should control diabetes at least as well as insulin in mild cases and, if used with insulin in the more severe ones, dramatic evidence of their effectiveness should be available.

I was very interested in Stowers'* paper on competition for tubular excretion between chlorpropamide and salicylates. Salicylates raised the level of chlorpropamide in plasma; this finding may have a bearing on Segarra's* work in tuberculous patients who were receiving both drugs.

Henry Ricketts* stressed the necessity for a uniform method of clinical investigation when drugs are to be compared. All the established principles of good treatment of diabetes must be followed before a new variable is introduced.

The precise incidence of the complications produced by chlorpropamide in therapeutic doses is not known. It must be emphasized that each complication should be most thoroughly investigated. The evaluation of the significance of complications in the older-age group is, of course, particularly difficult.

Concluding Remarks

I have approached this subject as a physiologist and not as a clinician. We have in chlorpropamide an effective agent that appears to be more potent than the two drugs previously used. Adequate dose-response curves are not yet available. Chlorpropamide is longer acting than tolbutamide and can be given in smaller doses, which should always be small and should never exceed 750 mg. per day. It is probable that 500 mg. or less would be adequate in most patients, and this amount would be safer. The safe therapeutic range of chlorpropamide is narrow, and great attention must be paid to this point; Goldner has emphasized this strongly. Blood levels should be determined as the ideal, since relatively small doses may sometimes cause high levels and small increases in dose may have a surprisingly great effect on blood levels. Chlorpropamide tends to accumulate, and hypoglycemia may be disturbing, particularly during the early period of adjustment. Patients should be watched very carefully, since delayed responses, independent of dose, are sometimes seen.

We must emphasize again and again the favorable effects of dietary restriction alone. This has been pointed out repeatedly and stressed by Duncan

* Elsewhere in this monograph.

and is illustrated again by a case presented by Tolstoi. Wherever possible, patients should be standardized on diet alone. If more specific therapy is needed, the insulin requirement of all patients should be determined. If an oral agent is then used, its insulin equivalent is thus made available. These are valuable data, and they should be obtained whenever possible.

Someone has observed that the patients who respond best to the oral agents are those who do not need them. There is, of course, a real need for these agents in the right cases, but it is becoming increasingly obvious that many patients are receiving these drugs who could and should be controlled by diet alone. As Dobson said, the oral agents are frequently a convenience and not a necessity. In ten years we shall know if this convenience will have been perfectly safe.

I have attempted to quote a number of the distinguished clinicians and I conclude by referring to the finding made by Alexander Marble* that chlorpropamide is an effective agent. Marble encountered a disturbing side effect in one patient of seventy-two, even with a relatively small dose of the drug. He believes, as I do, that there are many advantages in having a number of safe active oral agents available, and he stressed the fact that more exact clinical work on chlorpropamide is needed to determine its proper sphere of usefulness.

It is important that the national diabetes associations intensify their efforts at the present time to help physicians in the selection of cases and in development of an appreciation of the risks involved and the benefits to be obtained from the oral hypoglycemic agents.

Acknowledgments

I am indebted to my Toronto colleagues A. Sirek and O. Sirek, Donald Clarke, and Bernard Leibel for providing me with information in the preparation of this paper.

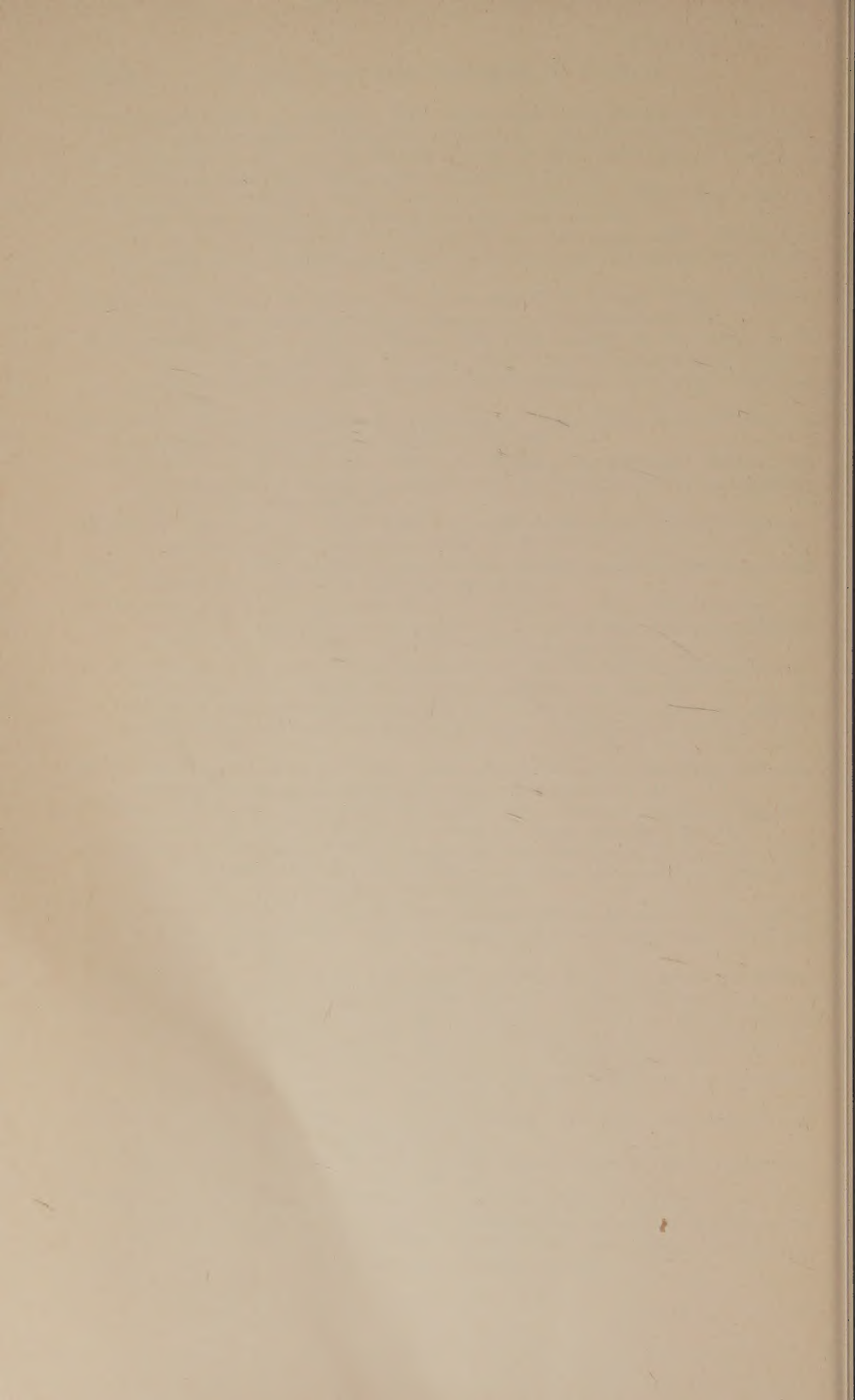
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